VOLUME 73 *March, 1956* NUMBER 3

THE AMERICAN REVIEW OF TUBERCULOSIS AND PULMONARY DISEASES

OFFICIAL JOURNAL OF AMERICAN TRUDEAU SOCIETY
MEDICAL SECTION OF NATIONAL TUBERCULOSIS ASSOCIATION

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ANNUAL MEETING
NEW YORK, NEW YORK, May 21-24, 1956

Published Monthly at Mount Royal and Guilford Avenues, Baltimore 2, Maryland, by the National Tuberculosis Association • Business Off., 1790 Broadway, New York 19, N. Y.

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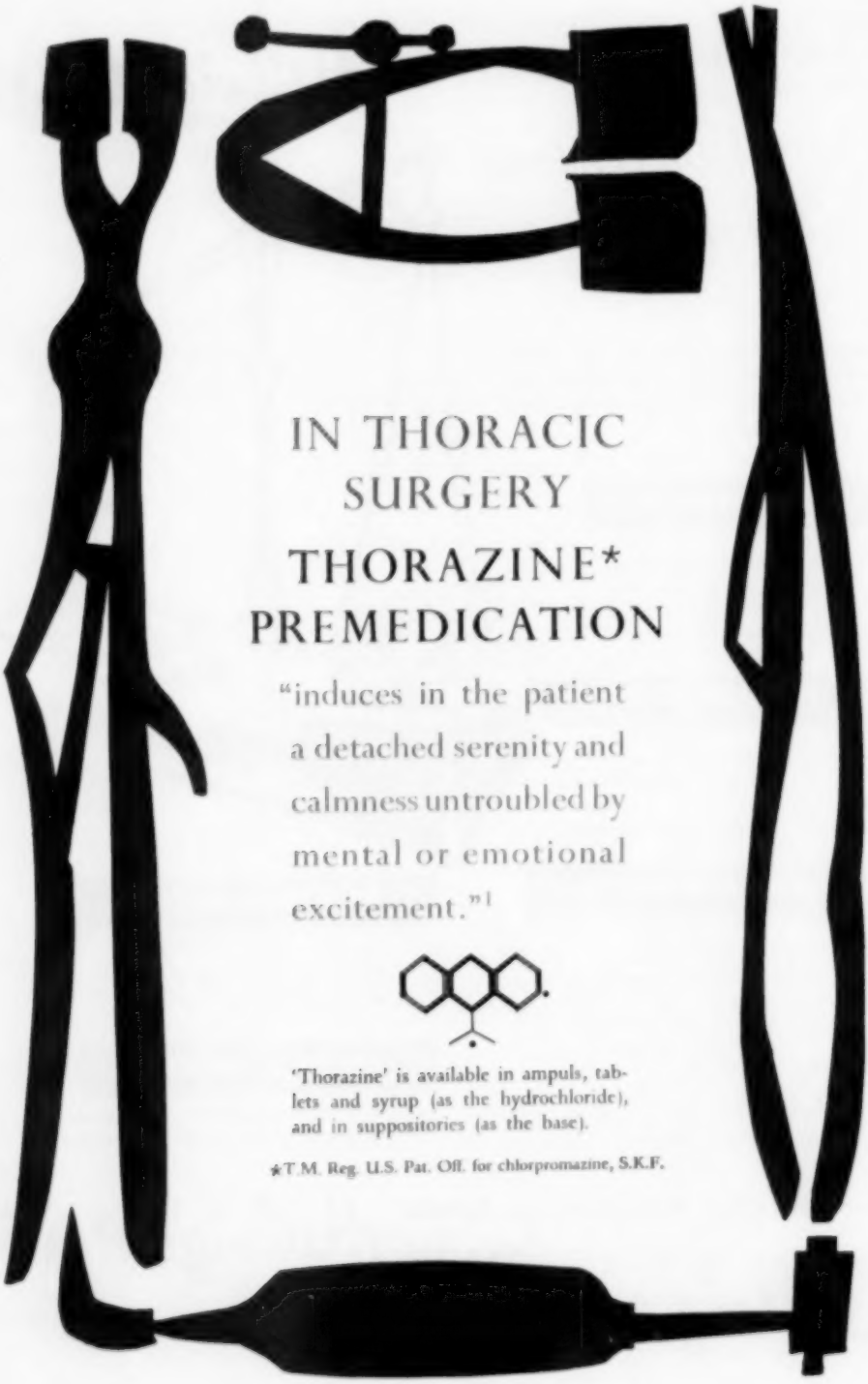

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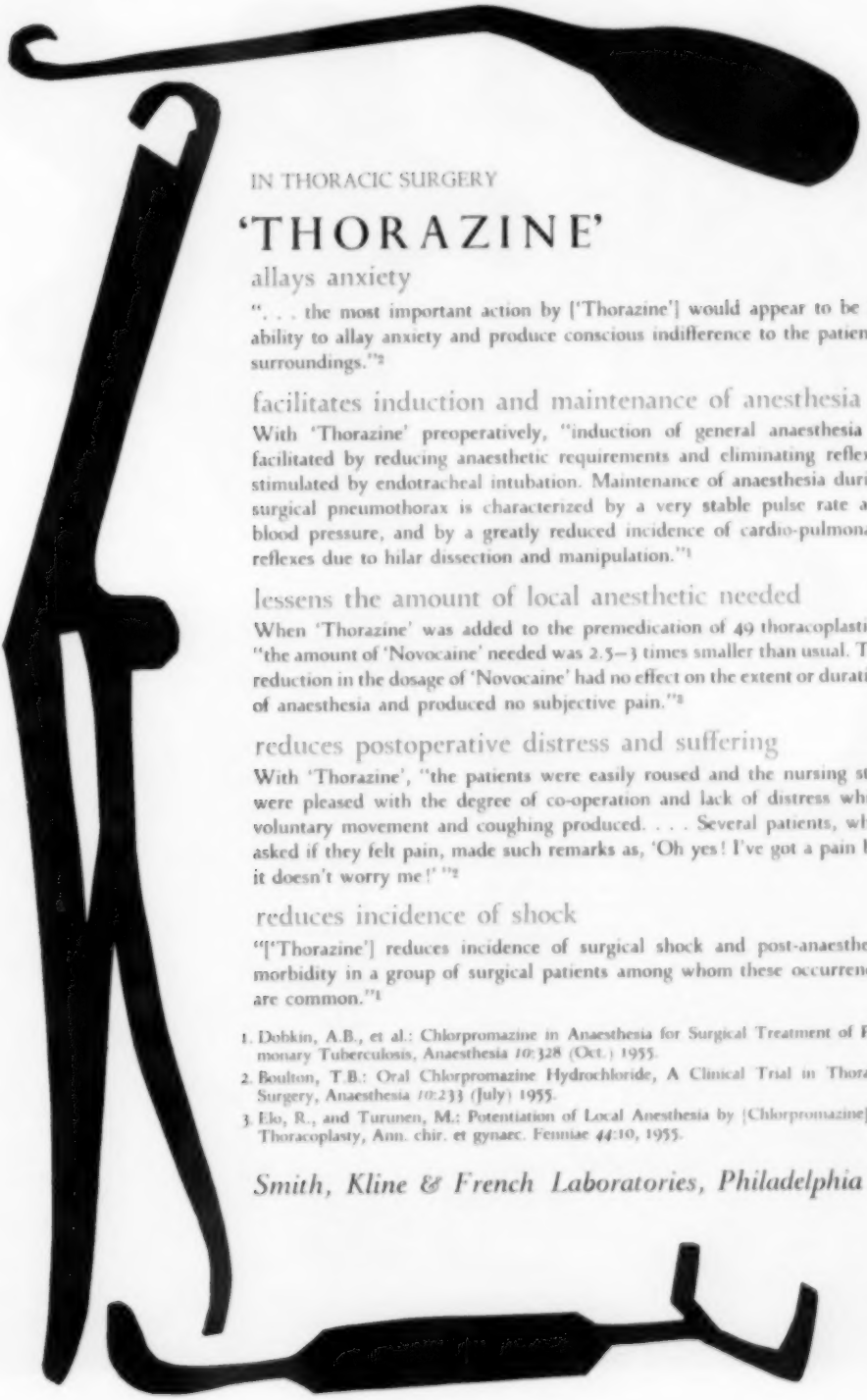

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3. Elo, R., and Turunen, M.: Potentiation of Local Anesthesia by [Chlorpromazine] in Thoracoplasty, Ann. chir. et gynaec. Fenniae *44*:10, 1955.

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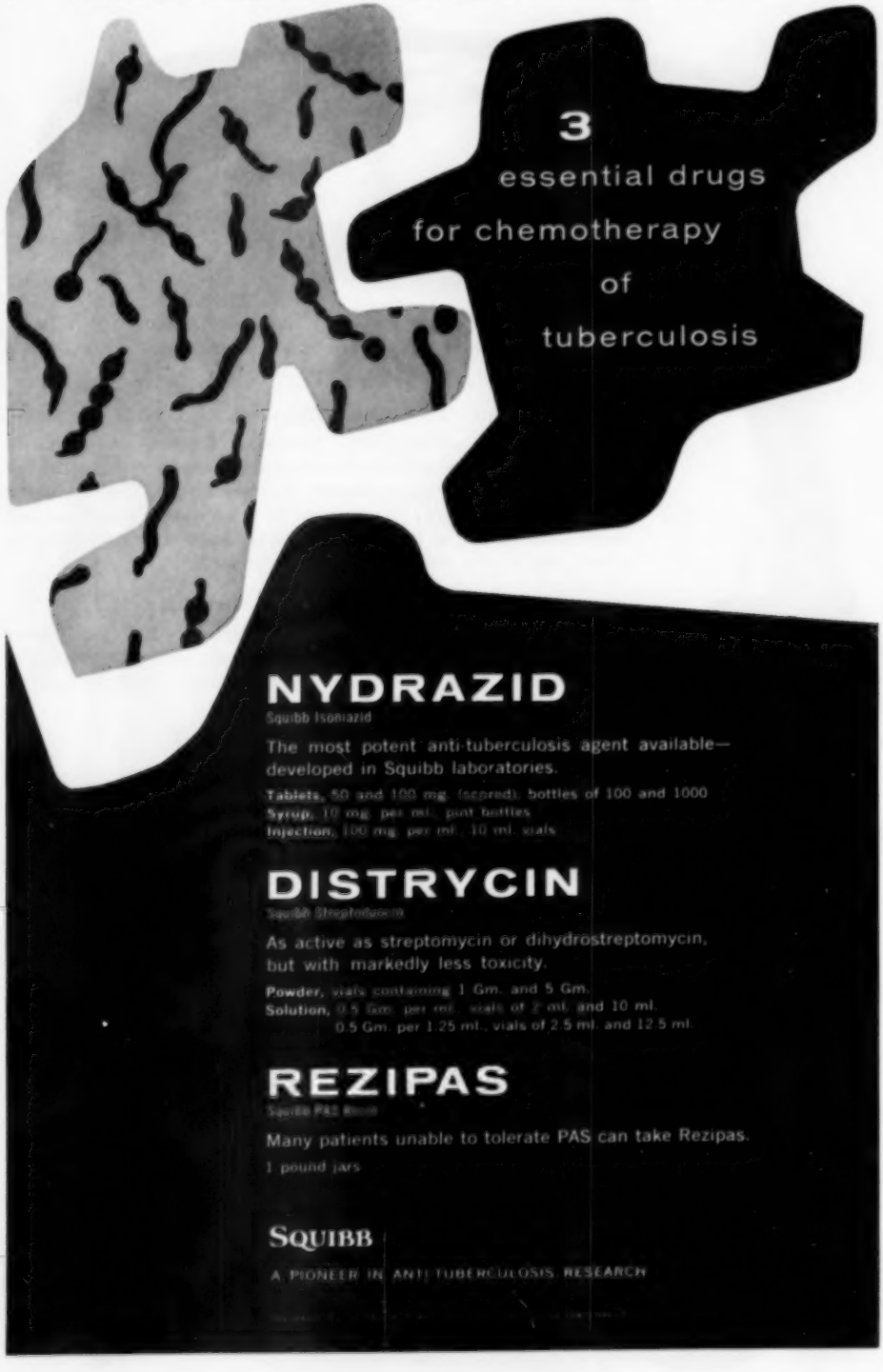
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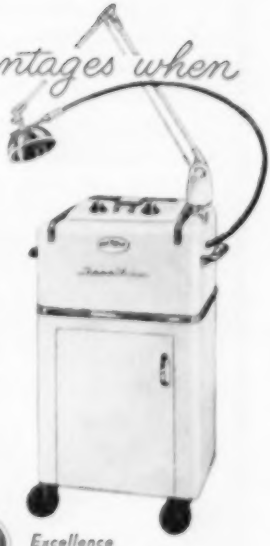

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1. *Rudin, L. N., and Jensen, J. R., C. M. Digest, XX: 7, 1953*
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Since publication of our paper entitled "Whereto Tuberculosis? The First Seven Years of the Antimicrobial Era, 1947–1953" in the October, 1955, REVIEW, attention has been called by the Central Bureau of Statistics of Norway to the need of correcting the figure for new cases reported during 1953. The Chief of the Demographic and Health Statistics Division of Norway points out that the original figure of 3,073 quoted included "recidives" (relapsed), namely, old or previously reported cases and that it should have been only 2,156.

Accordingly, when the latter smaller figure for the year 1953 is compared with the 3,719 new cases during the year 1947, it is seen that the reduction during those seven years amounted in number to 1,563 or by no less than 42 per cent—rather than the 17 per cent mentioned in the original article.

Likewise, for the year 1953 the community case fatality, namely, the ratio between the new cases reported, 2,156, and the tuberculosis deaths, 539, should now read 25 per cent. And the morbidity rate or ratio of new cases to the total population was 64 per 100,000 rather than 91—a lowering of 54 points or of 46 per cent since 1947.

The corrected figure for morbidity in 1953 permits now a better and interesting comparison of the great progress made in Norway as well as in the other Scandinavian countries. It is, therefore, worth stating that in 1953, as compared with 1947, the tuberculosis morbidity rate had fallen by 46 per cent in Norway, 57 per cent in Sweden, 53 per cent in Denmark, 38 per cent in Finland, and 49 per cent in Iceland.

The undersigned authors wish to acknowledge again the cooperative, cordial attention given our requests for information by the authorities of these progressive Scandinavian countries.

G. J. DROLET
A. M. LOWELL

December 7, 1955

THE AMERICAN REVIEW OF TUBERCULOSIS AND PULMONARY DISEASES

VOLUME 73 *March, 1956* NUMBER 3

EXPERIMENTAL AIR-BORNE INFECTION[1]

A Comparison of Different Masks in the Protection of Rabbits Against Inhalation Infection with Tubercle Bacilli

SAMUEL ABRAMSON[2]

With the technical assistance of Florence Richardson Feller

(Received for publication August 8, 1955)

INTRODUCTION

It has been demonstrated (1) that, when all of the air respired by rabbits exposed to an atmosphere laden with isolated or small units of virulent bovine tubercle bacilli passed through three or six layers of gauze (44 by 44 threads per square inch mesh), there was a 91 per cent reduction in the generation of gross primary pulmonary tubercles after a given time. This study permitted a certain amount of application to the problem of protection of human beings, since a gauze material in active use for human face masks (2) was tested in living persons under conditions of exposure to infection and subsequent development of disease that were both natural and quantitative. In these experiments the mask material provided a high degree of protection in spite of a greater degree of contagion than would be likely for exposed human beings. It was of interest to approach human conditions more closely through a study of the protective capacity of the identical material when incorporated into a face mask comparable in design and operation with the type commonly worn by human beings. The present report concerns itself with this problem and with the comparative protective value of an airtight respirator with felt filter or gauze filter.

Review of the Literature

The face mask was among the earliest techniques employed in attempting to protect human beings against the inhalation of air-borne pathogens. In view of the obvious importance of this problem, however, it is surprising that relatively few well-controlled experimental studies validating the protective value of the face mask have been reported. The obvious contradictions in much of the existing literature, the many variations in face masks recommended and in actual use, as well as the current conflicting opinions

[1] From the U. S. Department of Health, Education, and Welfare, Public Health Service, Tuberculosis Research Laboratory, Air-Borne Infection Unit, Henry Phipps Institute, Philadelphia, Pennsylvania.

[2] Present address: Department of Medicine, University of Chicago, Chicago 37, Illinois.

of their real value, reflect a lack of conclusive experimental evidence for their efficiency in preventing disease.

The majority of the older studies were devoted to a consideration of the capacity of various types of face masks to prevent escape of microorganisms from the upper respiratory tract of the wearer and subsequent contamination of his immediate environment. Certain recent studies have been concerned with the protection of individuals against the inhalation of bacterial aerosols (1, 3, 4). That the latter approach may be of greater value is suggested by the work of Hirshfeld and Laube (5), who concluded that it was impossible to construct a filter capable of preventing outward escape of all bacteria without making it so resistant to the passage of air that breathing through it was arduous, and, further, that it was difficult to fit a mask to the face so that air did not escape around it. The older studies are subject to criticism also because they failed to consider and enumerate the bacteria that remain suspended in the air for indefinite periods of time, viz., droplet nuclei as distinguished from droplets (6). Arnold (7) considered the role of droplet nuclei and, although he was primarily concerned with prevention of escape of bacteria from their source, stressed the importance of avoiding leakage around the edges of the face mask; he therefore urged the use of the face mask as a filter rather than as a deflector. Hirshfeld and Laube (5) demonstrated that, when an unmasked human being talks, he expels large numbers of bacteria into the air and that the surgical mask reduced but failed to eliminate direct spray contamination (droplets) and indirect contamination (droplet nuclei) under these conditions.

Rooks, Cralley, and Barnes (3) passed aerosols of droplet nuclei through various gauze materials mechanically at a rate comparable with the flow rates in human respirations. They demonstrated that six layers of gauze (42 by 42 strands), after twenty launderings, showed a 97 per cent bacterial filtering efficiency and at the same time a low resistance to the flow of air through them. They cited preliminary experiments which showed that bacteria tend to escape around the edges of the impermeable type of mask. Hence, they considered it likely that a mask with a high bacterial filtering efficiency but with a high resistance to air flow would allow escape of bacteria around the mask. Engelfried and Farrar (4) also worked with droplet nuclei and an apparatus for testing the bacterial filtering capacity of mask materials, and essentially confirmed the work of Rooks and associates by demonstrating high efficiencies for a variety of mask materials.

Attention to the concept of droplet nuclei (6) and to the role of particle size in penetration of infective agents to the region of the respiratory tract susceptible to the generation of lesions (8) is of special and decisive importance in any consideration of pulmonary tuberculosis (9, 10) or other infectious diseases which localize primarily in the pulmonary parenchyma.

MATERIALS AND METHODS

As the essential features of the method of quantitative air-borne infection used in these experiments have already been described and evaluated (1, 10), only brief mention of the principles need be made here. The apparatus used correlates well in construction and in the essentials of operation and performance with the one described by Lurie and co-workers (10). It represents a modification of the apparatus developed by Wells (11) and consists of three principal components connected in series: (*a*) an atomization device for transferring microorganisms from specially prepared aqueous suspension to suspension in air, mainly in the form of single units; (*b*) an infection chamber permitting the simultaneous exposure of six animals to inhalation of bacterial aerosol under conditions of safety and convenience; and (*c*) a baffled, gas-operated incinerating chimney and centrifugal exhaust fan placed at the end of the system to maintain a constant air flow through it, destroy the organisms after their passage through the infection chamber, and discharge the incinerated air to the outdoor atmosphere. In operation, constantly produced aerosol is carried by continuous venti-

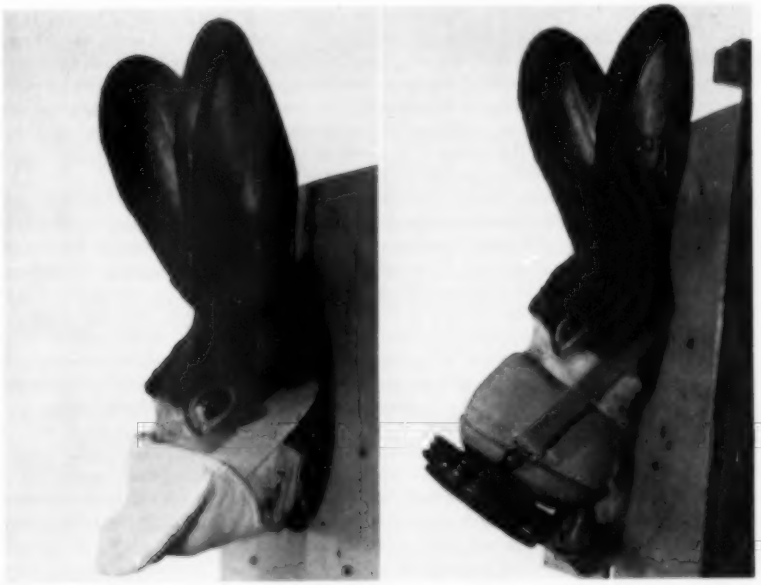

FIG. 1. (*Left*) Rabbit wearing experimental gauze face mask.
FIG. 2. (*Right*) Rabbit wearing experimental airtight respirator.

lation into and out of the infection chamber, and certain techniques facilitate the quantitation of dosage of microorganisms deliverable to the experimental animals via inhalation, within certain assessable limits (10). Young cultures of bovine tubercle bacilli of the Ravanel strain, grown on a modified Löwenstein solid medium (12), were employed in the preparation of the aqueous suspensions. In contrast to the techniques referred to above (10), it was found unnecessary, in this study, to filter the suspensions prepared for atomization. Centrifugation at 2,000 r.p.m. for ten minutes sufficed to yield a suspension in which the majority of the microorganisms were isolated; such few clumps as remained very rarely consisted of more than six bacilli as indicated by Ziehl-Neelsen stains of smears.

The rabbits were purchased from a single dealer and were of mixed sex and heterogeneous constitution. Each animal was subjected to a tuberculin test prior to use, and all proved to be negative. Within the limits of the method (10, 14), some degree of control over dosage of bacillary units[3] of tubercle bacilli inhaled by the rabbits in each experiment was exercised by selection of animals on a basis of comparable weights, suitable dilutions of the suspensions used for atomization, and proper timing of exposure periods.

The gauze face masks were fabricated by modifying the same gauze hoods used in an earlier study (1). The results detailed in the present report, therefore, are directly comparable in certain respects with the previous one. The gauze was six-layered and of 44 by 44 threads per square inch mesh. The portion of each gauze hood that extended upward and backward so as to cover completely the rabbit's head and neck (1) was removed, leaving only enough material to cover the nose and mouth and a small portion of the face adjacent to these parts. Two different sizes of mask were prepared in order to permit adjustment to natural variations in the animals. Each mask was equipped with an elastic band and adjustable buckle-type fastener to maintain it in position on the rabbit's face. In figure 1 is illus-

[3] This term refers to individual infective particles, each of which may contain one or several individual bacilli.

trated the fit of the face mask on a rabbit, as well as the features described below. It was desired to simulate as much as possible the anatomic relationships which exist when human beings wear face masks, and in such circumstances the face mask ordinarily fails to effect an airtight seal laterally on each side of the nose. Consequently, an inner, longitudinal pocket was sewn in the midline of the upper aspect of the rabbit face mask. This pocket was filled out by inserting short lengths of wood applicator sticks, thereby simulating the projecting human nose. In this manner the creation of a seal at the indicated areas was deliberately prevented and air leakage was consequently permitted.

It is apparent that in human beings the nostrils are oriented in a more or less downward direction, and the tip of the nose prevents actual contact between the mask itself and the margins of the nostrils. In the rabbit, however, the dorsum of the face is flat, and the nostril margins project forward and upward. To avoid the inevitable contact that would be effected between the rabbit nostrils and the cone-shaped mask, two lateral darts, commencing at the extremity and extending backward approximately one-half its length, were sewn into the experimental mask. This procedure narrowed the cone so as to limit the extension of the rabbit's face into the mask and thus, by producing a small space between the margins of the animal nostrils and the material of the mask, further simulated human use of face masks.

As leakage around the edges of the face mask is probably a factor which limits its efficiency, it was of interest to study a device that would eliminate this variable. For this purpose, a commercially available respirator[4] was subjected to experiment. The essential features of this device, designed originally for the protection of human beings against the inhalation of injurious dusts, include a soft rubber face piece which conforms to and makes an airtight seal with the face, a metal holder adaptable for maintaining a variety of filter disks in position, and synchronized inhalation and exhalation valves.

In order to test this respirator properly in rabbits, it was necessary to replace the standard face piece with a specially designed and fabricated modification which fitted the animal face. In figure 2 is illustrated the fit of the experimental respirator on a rabbit.

Two different filters were tested: (*a*) American Optical Company, Safety Products Division, Filter # R25, a chemically treated felt filter with gauze and cotton prefilter, rated by its manufacturer to protect against the inhalation of dusts as small as those having a mean particle size of 0.61 micron[5]; and (*b*) a filter fabricated from six layers of the same type of gauze as was used for the gauze face masks described above.

The # R25 felt filters used in these experiments were purchased on the open market. Unfortunately, following the fabrication of the gauze face masks through modification of the original gauze hoods, not enough material remained to permit its use for the filter disks. Therefore, gauze material of the same type (44 by 44 strands per square inch mesh) was purchased from the original supplier[6], washed once before initial use, folded into six layers, and cut to fit the circular filter holder of the respirator. Since the gauze filter disk was thinner than the felt filter, it was necessary to use a narrow, circular rubber gasket to effect an airtight seal between the retaining flanges of the filter holder and the periphery of the gauze filter. The correspondence between the rated protective capacity of the felt filter and the size range of tubercle bacilli is obvious, and it is also apparent that the use of the respirator with gauze filter was intended to provide a comparative animal test of the filtering efficiency of the gauze material with elimination of the leakage variable.

The various experiments were divided into three groups. In the first group, each experiment comprised the simultaneous exposure to inhalation of contaminated air of 3 control rabbits and 3 rabbits wearing gauze face masks; in the second group, each experiment involved the simultaneous exposure of 2 control rabbits, 2 rabbits wearing gauze face masks, and 2 rabbits wearing

[4] American Optical Company, Safety Products Division, Respirator # R200.

[5] The # R25 filter is not the most efficient type of filter offered by the manufacturer.

[6] Progressive Coat and Apron Manufacturing Co., 2701 N. Broad Street, Philadelphia, Pennsylvania.

respirators with felt filters; in the last group, each experiment included 2 control animals, 2 animals wearing respirators with felt filters, and 2 with respirators having gauze filters.

In the case of each experimental rabbit, the hair of the entire face, including the jaw, up to the line of the eyes, was closely clipped to eliminate the possibility of filtration by hair. This is illustrated in figures 1 and 2. Only the hair about the nose and mouth was removed from the control animals.

In each experiment the animals were exposed to contaminated air for a period of thirty minutes. Following each exposure period, the gauze face masks were examined, autoclaved, and, prior to use in a succeeding experiment, thoroughly washed and dried. After each use of the respirator, the filter was examined and discarded into a disinfectant solution prior to incineration, and the respirator itself was completely immersed in 70 per cent ethyl alcohol for four hours, after which it was thoroughly rinsed in tap water and air-dried.

In the case of animals which died within a short time following an exposure period, except where otherwise noted, the lungs were removed and cultured quantitatively for tubercle bacilli according to a method previously described (1); in each case appropriate control animals were sacrificed and their lungs cultured at the same time. This practice of sacrifice and lung culture was applied also in the instances when, for any reason, there was question concerning the future survival of an animal. Otherwise, the animals were allowed to survive the infection period until the controls developed a strong tuberculin reaction, at which time all of the animals exposed simultaneously during a given experiment were sacrificed. In practice, this usually meant that the majority of the animals were sacrificed within four to five weeks following infection. At this time complete autopsies were performed on each animal, and the entire lung was removed and inflated with 10 per cent neutral formalin. After immersion in this fluid for several weeks, careful counts were made of the total number of gross primary pulmonary tubercles by excision of each individual focus. Thus, the criterion of protection was the comparative number of gross primary tubercles discoverable in the lungs of control and experimental animals.

It was not possible in each experiment to reproduce precisely bacterial aerosols having identical populations. This was reflected in variations, from experiment to experiment, in numbers of pulmonary tubercles observed among control animals, on the one hand, and experimental animals on the other (tables 1–3). It was not deemed valid, therefore, to average these numbers for all of the rabbits grouped in these two general categories. Instead, it seemed that the most objective assessment of protection would be derived by calculating the degree of protection demonstrated in individual experiments, in which given control and experimental animals were exposed to identical conditions, and then presenting the average protection expressed as a percentage.

RESULTS

The distribution of the animals in the first series of experiments is indicated and the results are tabulated in table 1. Comparison between the average number of gross pulmonary tubercles in the control and experimental animals in indi-

TABLE 1

EFFICIENCY OF GAUZE FACE MASKS IN PROTECTING RABBITS AGAINST THE INHALATION OF TUBERCLE BACILLI

Date of Experiment	Control		Gauze Face Mask		
	Rabbbit Number	Number of Gross Primary Pulmonary Tubercles	Rabbit Number	Number of Gross Primary Pulmonary Tubercles	Per Cent Protection
1/30/52 Part *A*	M-137	33	M-140	6	73.5
	M-138	29	M-141	17*	
	M-139	40	M-142	4	
1/30/52 Part *B*	M-143	24	M-146	†	45.2
	M-144	44	M-147	†	
	M-145	25	M-148	17	
2/1/52	M-135	76	M-136	18	69.9
	M-149	79	M-150	34	
	M-151	57	M-152	12	
2/5/52	M-158	53	M-157	†	81.4
	M-156	78	M-155	12	
	M-153	22	M-154	7	
2/12/52	M-166	22	M-169	9	81.7
	M-171	42	M-172	28‡	
	M-173	26	M-174	2	
2/15/52	M-188	§	M-187	‖	50.8
	M-190	31	M-189	4	
	M-191	34	M-192	28	

* Possible dislodgment of face mask during exposure period; number of tubercles included in average.

† Died shortly after exposure; eliminated from experiment.

‡ Rabbit chewed two holes through fabric of face mask during exposure period; through these holes contaminated air could have been inhaled directly; number of tubercles excluded from calculations.

§ Sacrificed seven days after exposure; 431 tubercle bacilli cultured from lungs.

‖ Died seven days after exposure; 208 tubercle bacilli cultured from lungs.

vidual experiments shows a range of 45.2 to 81.7 per cent protection, with an average of 67.1 per cent. It is evident that in six of six experiments clear-cut protection was demonstrated. The lungs of rabbits employed in a typical experiment (February 1, 1952) are illustrated in figure 3. During the exposure period in another experiment (February 12, 1952), rabbit M-172 chewed several holes through the fabric of the mask. It is likely that some bacilli passed directly through these openings, by-passing retention in the mask, thus accounting for the fact that the animal in question developed an inordinate number of tubercles as compared with other masked animals exposed at the same time. Hence, the

TABLE 2

RELATIVE EFFICIENCY OF GAUZE FACE MASKS AND RESPIRATORS WITH FELT FILTERS IN PROTECTING RABBITS AGAINST THE INHALATION OF TUBERCLE BACILLI

Date of Experiment	Control		Gauze Face Mask			Respirator with Felt Filter		
	Rabbit Number	Number of Gross Primary Pulmonary Tubercles	Rabbit Number	Number of Gross Primary Pulmonary Tubercles	Per Cent Protection	Rabbit Number	Number of Gross Primary Pulmonary Tubercles	Per Cent Protection
3/12/52	M-224 M-220	86 †	M-205 M-218	14 13	84.3	M-221	*	
3/19/52	M-226 M-211	13 ‡	M-213 M-216	4 0	69.2	M-203 M-212	0 ‡	100.0
3/20/52	M-222 M-207	§ 34	M-214 M-219	6 5	83.8	M-215 M-217	‖ 0	100.0
3/21/52	M-230 M-231	76 106	M-210 M-223	19 8	85.2	M-204 M-229	0 3#	89.5
4/4/52	M-228 M-232	46 18	M-208 M-238	8 9	73.4	M-233 M-234	0 0	100.0
4/9/52	M-241 M-244	** 17	M-206 M-209	2 1	91.2	M-239 M-225	†† 0	100.0
4/10/52	M-240 M-250	19 22	M-236 M-242	3 0	92.7	M-243 M-258	0 4	90.2
4/16/52	M-263 M-264	34 11	M-261 M-262	0 0	100.0	M-260 M-265	1 2‡‡	93.3

* Died shortly after exposure; lungs cultured twenty-four hours later; no tubercle bacilli recovered.

† Sacrificed one day after exposure; 585 tubercle bacilli cultured from lungs.

‡ Sacrificed three days after exposure; no tubercle bacilli demonstrated in lungs by culture.

§ Sacrificed two days after exposure; 308 tubercle bacilli cultured from lungs.

‖ Sacrificed two days after exposure; no tubercle bacilli cultured from lungs.

Questionable whether respirator remained in proper position throughout exposure period. Animal included in calculations.

** Sacrificed one day after exposure; 86 tubercle bacilli cultured from lungs.

†† Sacrificed one day after exposure; no tubercle bacilli cultured from lungs.

‡‡ Determined by careful inspection and palpation.

indicated animal has been excluded from the calculations. Rabbit M-187, masked during exposure (February 15, 1952), died on the seventh day following infection. Culture of the lungs yielded 208 bacillary units of tubercle bacilli, as compared with 431 bacillary units cultured from the lungs of control rabbit M-188, exposed simultaneously and sacrificed at the same interval following infection.

In table 2, the results of the second group of experiments are detailed. Com-

TABLE 3

RELATIVE EFFICIENCY OF RESPIRATORS WITH FELT FILTERS AND GAUZE FILTERS IN PROTECTING RABBITS AGAINST THE INHALATION OF TUBERCLE BACILLI

Date of Experiment	Control		Respirator with Felt Filter			Respirator with Gauze Filter		
	Rabbit Number	Number of Gross Primary Pulmonary Tubercles	Rabbit Number	Number of Gross Primary Pulmonary Tubercles	Per Cent Protection	Rabbit Number	Number of Gross Primary Pulmonary Tubercles	Per Cent Protection
4/17/52	M-268	4	M-266	0	100.0	M-267	25	0
	M-269	*	M-273	0		M-270	21	
4/21/52	M-271	65	M-278	0	100.0	M-281	64	0
	M-276	50						
4/22/52	M-274	71	M-275	0	100.0	M-288	63	0
	M-277	58	M-280	0		M-287	†	
4/23/52	M-292	88	M-283	3‡	96.6	M-294	101	0
	M-284	§	M-296	ǂ		M-295	47	
4/24/52	M-245	36	M-291	0	98.8	M-289	110	0
	M-272	49	M-297	1		M-290	52	
4/25/52	M-285	26	M-298	0	100.0	M-299	17	0
	M-301	48	M-302	0		M-300	47	

* Died twenty-two days after exposure, due to cause unrelated to tuberculosis; failure to find gross pulmonary tubercles possibly related to pneumonic changes associated with severe primary liver disease.

† Died four days after exposure; eliminated from experiment.

‡ Questionable whether respirator remained in proper position throughout exposure period. Animal included in calculations.

§ Died during exposure period; eliminated from experiment.

ǂ Died three days after exposure; eliminated from experiment.

paring the average number of gross pulmonary tubercles which developed in the control rabbits with the number in the masked rabbits shows a range, in the individual experiments, of 69.2 to 100 per cent protection, with an average of 84.9 per cent. Four of the gauze face-masked animals were so protected that no gross pulmonary tubercles developed; in comparable control rabbits, exposed at the same time, from eleven to thirty-four pulmonary tubercles were generated.

It will be evident from an examination of both tables 1 and 2 that in fourteen of fourteen experiments, the gauze face masks provided unquestionable protection of a considerable degree. The per cent protection in the individual experiments ranged from 45.2 to 100 per cent, with an average protection of 77.3 per cent. In the case of the respirator with felt filter (table 2), the per cent protection ranged from 89.5 to 100 per cent, with an average of 96.1 per cent. Among 11 surviving rabbits exposed while wearing respirators with felt filters, 7 (64 per cent) failed to develop any gross tubercles, whereas comparable control animals generated from 13 to 106 tubercles. The respirator provided apparently

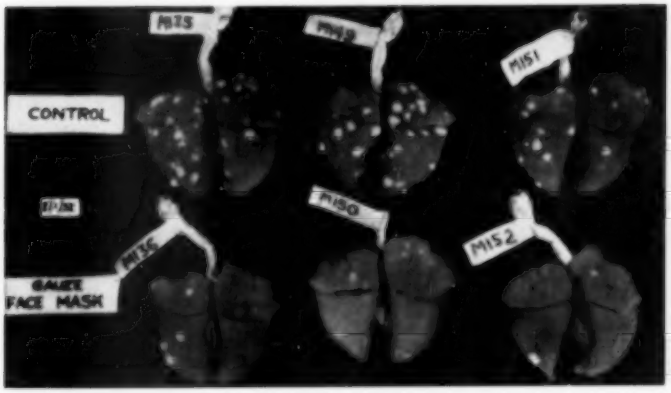

FIG. 3. Lungs of control and gauze face masked rabbits at autopsy thirty-two days after simultaneous exposure to inhalation of bovine tubercle bacilli. In the controls (M-135, M-149, M-151), 76, 79, and 57, gross primary tubercles, respectively, were counted; while in the experimental group (M-136, M-150, M-152), 18, 34, and 12 gross primary tubercles, respectively, were found.

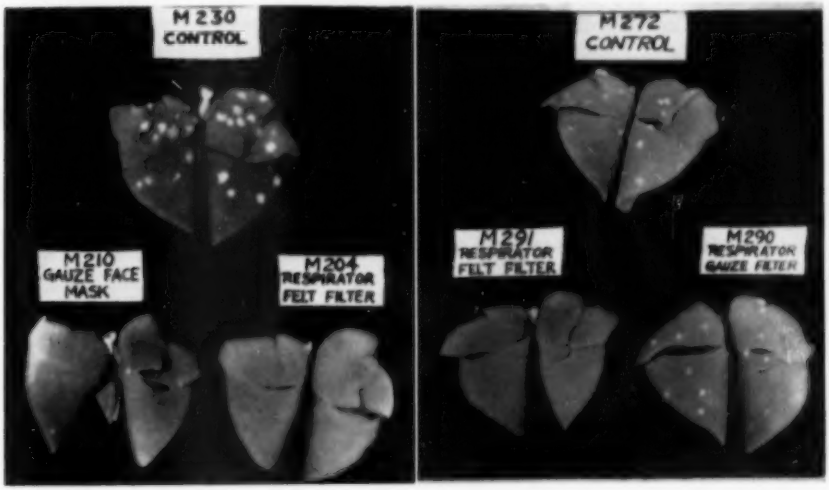

FIG. 4. (*Left*) The lungs of control and experimental rabbits at autopsy twenty-eight days after a simultaneous exposure to inhalation of bovine tubercle bacilli. The control rabbit (M-230) had 76 gross primary pulmonary tubercles, the gauze face masked animal (M-210) had 19 tubercles, and the rabbit who wore a respirator with felt filter (M-204) had no gross pulmonary tubercles.

FIG. 5 (*Right*) The lungs of control and experimental rabbits at autopsy twenty-eight days after a simultaneous exposure to inhalation of bovine tubercle bacilli. The control rabbit (M-272) had 49 gross primary pulmonary tubercles, the rabbit with respirator and felt filter (M-291) had no gross pulmonary tubercles, and the animal with respirator and gauze filter (M-290) had 52 gross primary pulmonary tubercles.

complete protection in these cases. Four other rabbits tested with the respirator and felt filter (M-221, M-212, M-215, and M-239) died or were sacrificed within one to three days following exposure; no tubercle bacilli were demonstrable in the lungs of these rabbits by culture. By contrast, 585, 308, and 86 bacillary units of tubercle bacilli, respectively, were cultured from the lungs of comparable control rabbits who had been exposed at the same time. An example of the relative degree of protection provided by the gauze face mask and the respirator with felt filter is illustrated in figure 4.

In table 3 are shown the results obtained in the last group of experiments, which involved additional controlled tests of the respirator with felt filter as well as a direct comparison between the felt and gauze filter when used with the respirator. In six individual experiments, the protection afforded by the respirator with felt filter ranged from 96.6 to 100 per cent, with an average of 99.1 per cent. Of the 10 rabbits who wore this device, 8 (80 per cent) failed to generate any gross pulmonary tubercles, in contrast to comparable control animals in which 4 to 71 tubercles developed. Concerning the rabbit (M-283) in which 3 gross tubercles were found in the lungs, it is doubtful that all of the air respired during the infection period actually passed through the felt filter. The animal struggled during this time and, near the end of the infection period, succeeded in changing the position of the respirator, possibly breaking the airtight seal. Nonetheless, the calculations include the number of pulmonary tubercles found in this animal. As to the respirator with gauze filter, it will be evident from table 3 that in three of six individual experiments, the experimental rabbits averaged more gross pulmonary tubercles than the controls, and in the remaining experiments there was obviously no significant difference between control and experimental animals. This warrants the conclusion that the gauze filters, when used with the respirators, provided no protection for these rabbits under the given conditions. An example of the difference in protective effect between the felt and gauze filters is illustrated in figure 5.

Data obtained from each of the three groups of experiments are presented in summary in table 4.

TABLE 4

EFFICIENCY OF DIFFERENT MASKS IN PROTECTING RABBITS AGAINST THE INHALATION OF TUBERCLE BACILLI

Type of Mask	Number of Experiments	Number of Animals		Average Per Cent Protection	Per Cent of Apparent Complete Protection*
		Control	Experimental		
Gauze face mask	14	29	29	77.3	13.8
Respirator with felt filter	13	22	21	97.6	71.4†
Respirator with gauze filter	6	10	10	0.0	0.0

* Per cent of animals showing complete absence of gross pulmonary tubercles.

† In 4 additional animals in this category, no tubercle bacilli were recovered on individual culture of the lungs.

DISCUSSION

It has been demonstrated in this study that gauze face masks, applied to rabbits so as to simulate closely human conditions, prevented the inhalation of sufficient tubercle bacilli to cause a 77 per cent reduction in the generation of gross pulmonary tubercles. It has been shown, further, that a respirator, which maintained an airtight facial contact, provided almost complete protection when used with a chemically treated felt filter, but offered no protection when used with a gauze filter.

As already indicated, the results obtained with the gauze face masks are comparable with the results of an earlier experiment (1) which demonstrated that the identical gauze material, applied in the form of a hood through which all of the respired air passed, removed 91 per cent of the pathogenic units of tubercle bacilli. The fact that the gauze face masks, fabricated by modification of these gauze hoods, were 14 per cent less protective than the latter may be attributed, in part, to leakage around the edges of the face masks. In the case of the gauze hoods (1) it is likely that a certain amount of rebreathing of air occurred. It is reasonable to assume that any rebreathing that occurred with the gauze face masks was minimal.

The achievement of a 77 per cent reduction in development of gross pulmonary disease by use of gauze face masks represents considerable protection especially in view of the obvious opportunity for some air leakage. Moreover, this degree of protection was provided in spite of an intensity of contagion that would not be likely to exist for human beings exposed under natural conditions. This is attested by the fact that the unprotected rabbits developed multiple primary tubercles (tables 1–3), whereas it is well known that human primary tuberculosis originates usually as a single pulmonary focus. However, direct extrapolation of these data to human beings should be made with great care. Although a remarkable similarity exists between rabbits and man with respect to the host-parasite relationship following natural infection with tubercle bacilli (13), the dynamics in the two respiratory tracts are not identical. Hence, there is no certainty that the pattern of protection observed in the animal studies reported here has an exact counterpart in human beings who employ these protective devices. Nonetheless, the data strongly suggest that, in so far as animal studies permit application to human conditions, there is good reason to expect considerable protection against air-borne tubercle bacilli in the dangerous particle size range from proper use of face masks by exposed human beings. It is pertinent, therefore, to emphasize again the specific conditions under which these results were obtained. Although there was definite opportunity for inward leakage of infected air laterally on each side of the simulated nose, and the results suggest strongly that such leakage indeed took place, the masks nevertheless fitted quite snugly around the face and under the jaws of the rabbits. By contrast, it is a matter of common observation that the fit of face masks worn by human beings frequently permits openings at various contact points. It is obvious that, if the maximum benefit is to be derived from the use of this technique, the gauze face mask should be used as a *filter* and not merely as a *deflector* (*see also* (1, 2, 7)).

The importance of eliminating leakage in improving the efficiency of the face mask is clear. However, it is possible that any physical barrier which reduces sufficiently the number of pathogenic particles available for actual deposition in the alveoli may be of value in preventing the development of clinical disease. There is some similarity between the resistance of human beings to mammalian tubercle bacilli and that of rabbits to strains of human tubercle bacilli. It was demonstrated (14) that even in susceptible rabbits only a fraction of the human bacilli that reached the terminal air passages induced the formation of visible tubercles. Nevertheless, when such animals inhaled a certain number of these bacilli they developed a variable and often extensive disease, in contrast to the absence of tuberculosis in resistant animals exposed under the same conditions. This provided a clear analogy to the presence or absence of active tuberculosis in man infected with tubercle bacilli. It seems reasonable that evidence for the concept of prevention of clinical disease by reducing the number of inhaled organisms below a critical level may be obtained by exposing control and masked rabbits, of known susceptibility, to quantitative air-borne infection with human tubercle bacilli, and comparing the generation of pulmonary lesions in the two groups. This remains to be done.

It is generally believed that wetting of a fabric mask reduces its filtering efficiency. However, definitive experimental proof of the effect of moisture on filtering efficiency is apparently unavailable. Although the presence or absence of moisture in the gauze face masks was noted following their use in these experiments, no consistent effect on filtering capacity was found and the details are therefore not included in this report.[7] Further work is needed to clarify the possible importance of this aspect of the technique.

The difference between the protective effects of the respirator, with felt filter on the one hand and with gauze filter on the other, requires consideration. It is necessary to state that the animals encountered greater resistance to inhalation with the respirator than with the simple gauze face mask. It was observed that this reduced respiration frequency and compelled a deeper inhalation effort. Neither the tidal air nor the respiratory frequency of the rabbits employed in the various experiments was measured. Physiologically, however, a deeper inhalation effort may increase the inspiratory flow rate. Brown (15) demonstrated that retention of particles in the lungs increased with decreasing respiration frequency. Landahl and Herrmann (16) came to the same conclusion and stated, further, "When the inspiratory flow rate is increased for a constant breathing pattern the retention increases." This they ascribed, in part, to penetration of air and particles to smaller respiratory passages. Furthermore, Landahl and associates (17) found that the percentage of rentention of particles increased with increased holding time of an air sample in the lungs. It is obvious that holding time in-

[7] The felt filters were uniformly dry after use in these experiments. Respirators with felt filters, identical with those under animal test except for the face pieces, were worn by laboratory personnel. No wetting of the filters occurred following uninterrupted wear for periods as long as four hours. This protection against wetting is ascribable to a valve system which seals off the filter during exhalations.

creases with decreased respiration frequency. Hence, in these experiments the altered breathing pattern of the rabbits apparently favored increased alveolar retention following particle penetration of gauze material having inferior filtering capacity under these conditions. It is clear that the respirator imposed a more rigorous test upon the filter materials than did the simple face mask and that the felt filters, used under exactly the same conditions as the gauze filters, displayed a high capacity to prevent disease.

The effectiveness of the gauze material when used in the form of a simple face mask, and its failure when employed as a filter disc in the respirator may be ascribable, in part, to the differences in size of the surface areas of these applications of the material, as well as to variations in pressure gradients of the air passing through them. In the case of the face mask, the gauze material presented a larger filter area, and opportunity was afforded for unimpeded air flow through deliberate leakage points. The gauze filter discs, on the other hand, presented a smaller filter area and were used under conditions in which all the inhaled air passed through them in one direction. It is likely, therefore, that the air flow rates per unit surface area were greater in the case of the gauze filter discs than in the gauze face masks. Although this would favor increased particle retention through increased impingement upon the fibers of the filter, the slippage effect was probably very high for the gauze filter discs under these conditions. Thus, the slippage effect (penetration of the filter following initial, temporary retention) would constitute the simplest explanation for the apparent inefficiency of the gauze material when used as a filter in the respirator.

Rooks and associates (3) found that maximum bacterial filtering efficiency of gauze material is reached after twenty periods of laundering. As stated earlier in this paper, insufficient material from a previous experiment (1) was available for preparation of the gauze filter discs, and these were prepared as indicated and used only once. There was no evidence from the earlier experiments, however, that as many as six launderings had any effect on the filtering efficiency of the material. It may be inferred, therefore, that the gauze material used for fabricating the filter discs, identical in type and source with that used in the earlier study, was, for all practical purposes, identical in other characteristics as well. It is of further pertinence to state that repeated use, with intervening laundering, of the gauze face masks used in the current study, gave no detectable increase in filtering efficiency.

SUMMARY

Under given experimental conditions, rabbits wearing gauze face masks, comparable in design and application with a type commonly worn by human beings, were protected against the inhalation of 77 per cent of the pathogenic tubercle bacilli present in the air of their environment. The importance of employing these masks as *filters* rather than as *deflectors* is emphasized. It is inferred that the gauze face masks might reduce the number of pathogenic particles available for actual deposition in the alveoli sufficiently to be of value in the prevention of clinical disease. Neither various degrees of wetting following use, nor

repeated laundering, appeared to have a consistent effect upon the protective capacity of the gauze face masks.

A commercially available airtight respirator, with chemically treated felt filter, was 97.6 per cent efficient in protecting rabbits under comparable conditions of intense exposure to air-borne infection. A gauze filter, employed in the same respirator, failed to provide any protection. The depth and frequency of respiration apparently played a significant role in influencing the relative efficiency of these protective devices.

SUMARIO

Infección Aerógena Experimental: Comparación de Diversas Máscaras para la Protección de los Conejos contra la Infección por Inhalación de Bacilos Tuberculosos

En ciertas condiciones de experimentación, los conjeos que llevaban máscaras faciales de gasa, comparables en diseño y aplicación a las de una clase usada por seres humanos, quedaron protegidos contra la inhalación de 77 por ciento de los bacilos tuberculosos patógenos que había en el aire del medio ambiente. Recálcase la importancia de emplear esas máscaras como *filtros* más bien que como *desviadores*. Infiérese la posibilidad de que las máscaras de gasa reduzcan el número de partículas patógenas disponibles para depósito en los alvéolos lo suficiente para que resulte de valor en la prevención de la enfermedad clínica. Ni el humedecimiento en varios grados después de usarlas ni los lavados repetidos parecieron ejercer efecto constante sobre la capacidad protectora de las máscaras.

Un respirator hermético ya de venta, provisto de filtro de fieltro tratado químicamente, mostró una eficacia de 97.6 por ciento para proteger a los conejos en condiciones comparables de intensa exposición a infección aerógena. Un filtro de gasa, empleado en el mismo respirador, no ofreció la menor protección. La profundidad y la frecuencia de la respiración desempeñaron aparentemente un papel importante en lo tocante a afectar la relativa eficacia de estos artefactos protectores.

RESUME

Infection expérimentale transmise par l'air. Comparaison de différents masques dans la protection des lapins contre l'innoculation par inhalation de bacilles tuberculeux

Dans certaines conditions expérimentales, des lapins porteurs d'un masque facial en gaze, comparable au masque utilisé habituellement par l'homme, ont été protégés contre l'inhalation de bacilles tuberculeux pathogènes présents dans l'air ambiant dans la proportion de 77 pour cent. L'accent est mis sur l'importance du port de ces masques à titre de *filtres* plutôt que de "*déviateurs*." La possibilité de réduire le nombre de particules pathogènes susceptibles de se déposer dans les alvéoles par leur rétention dans le masque facial en gaze peut être suffisante et favoriser la prévention de la forme clinique de l'affection. Divers degrés de mouillage ou de lessivage après usage n'ont pas paru avoir un effet uniforme sur la capacité de protection du masque facial en gaze.

On trouve dans le commerce un respirateur imperméable à l'air constitué par un filtre en feutre traité chimiquement, qui a une efficacité de 97.6 pour cent dans la prévention de l'infection du lapin soumis à une exposition d'intensité comparable à l'infection transmise par l'air. Un filtre en gaze, utilizé dans le même respirateur, n'a donné aucune protection. La profondeur et la fréquence de la respiration ont paru jouer un rôle important dans l'efficacité relative de ces appareils de protection.

Acknowledgments

The writer is indebted to Dr. Max B. Lurie for extending many of the facilities of his laboratory, and for helpful criticism of this manuscript.

Grateful acknowledgment is made to Mr. Laurence R. Crisp, Chief, Instrument Section, National Institutes of Health, Public Health Service, U. S. Department of Health, Education, and Welfare, Bethesda, Maryland, for the excellent design and construction of the apparatus for quantitative air-borne infection; and to the Safety Products Division, American Optical Company, Southbridge, Massachusetts, for fabricating and supplying the experimental respirators used in this study. Miss Louise Pizzaia, of the staff of the Henry Phipps Institute, constructed the model of a rabbit head that was used to mold the rubber face pieces of the experimental respirators.

REFERENCES

(1) Lurie, M. B., and Abramson, S.: The efficiency of gauze masks in the protection of rabbits against the inhalation of droplet nuclei of tubercle bacilli, Am. Rev. Tuberc., 1949, *59*, 1.

(2) McNett, E. H.: The face mask in tuberculosis, Am. J. Nursing, 1949, *49*, 32.

(3) Rooks, R., Cralley, L. J., and Barnes, M. E.: Hospital masks: Their bacterial filtering efficiency and resistance to air flow; a comparative study, Pub. Health Rep., 1941, *56*, 1411.

(4) Engelfried, J. J., and Farrar, F. W.: A study of surgical masks, U. S. Nav. Med. Bull., 1948, *41*, 524.

(5) Hirshfeld, J. W., and Laube, P. J.: Surgical mask, an experimental study, Surgery, 1941, *9*, 720.

(6) *a*. Wells, W. F.: On air-borne infection: II. Droplets and droplet nuclei, Am. J. Hyg., 1934, *20*, 611.

b. Wells, W. F., and Stone, W. R.: On air-borne infection: III. Viability of droplet nuclei infection, Am. J. Hyg., 1934, *20*, 619.

(7) Arnold, L.: A new surgical mask: A bacteriologic air filter, Arch. Surg., 1938, *37*, 1008.

(8) Sonkin, L. S.: The role of particle size in experimental air-borne infection, Am. J. Hyg., 1951, *53*, 337.

(9) Wells, W. F., Ratcliffe, H. L., and Crumb, C.: On the mechanics of droplet nuclei infection: II. Quantitative experimental air-borne tuberculosis in rabbits, Am. J. Hyg., 1948, *47*, 11.

(10) Lurie, M. B., Heppleston, A. G., Abramson, S., and Swartz, I. B.: An evaluation of the method of quantitative air-borne infection and its use in the study of the pathogenesis of tuberculosis, Am. Rev. Tuberc., 1950, *61*, 765.

(11) Wells, W. F.: On the mechanics of droplet nuclei infection: I. Apparatus for the quantitative study of droplet nuclei infection of animals, Am. J. Hyg., 1948, *47*, 1.

(12) Lurie, M. B.: The fate of BCG and associated changes in the organs of rabbits, J. Exper. Med., 1934, *60*, 163.

(13) *a*. Lurie, M. B.: Heredity, constitution and tuberculosis: An experimental study, Am. Rev. Tuberc., 1941, Supplement to vol. 44, 1.

b. Lurie, M. B., and Abramson, S.: Reproduction of human ulcerative pulmonary tuberculosis in rabbits by quantitative natural air-borne contagion, Proc. Soc. Exper. Biol. & Med., 1948, *69*, 531.

(14) Lurie, M. B., Abramson, S., and Heppleston, A. G.: On the response of genetically resistant and susceptible rabbits to the inhalation of human type tubercle bacilli and the nature of resistance to tuberculosis, J. Exper. Med., 1952, *95*, 119.

(15) Brown, C. E.: Studies in dust retention, III. Factors involved in the retention of inhaled dusts and fumes by man, J. Indust. Hyg. & Toxicol., 1931, *13*, 285.

(16) Landahl, H. D., and Herrmann, R. G.: On the retention of air-borne particulates in the human lung, J. Ind. Hyg. & Toxicol., 1948, *30*, 181.

(17) Landahl, H. D., Tracewell, T. N., and Lassen, W. H.: Retention of air-borne particulates in the human lung: III, A.M.A. Arch. Ind. Hyg., 1952, *6*, 508.

LOBAR VENTILATION IN MAN[1,2,3]

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(Received for publication May 25, 1955)

INTRODUCTION

It has been known for many years that the distribution of inspired gas within the lungs of man is not uniform (1). Anatomically this nonuniform ventilation might be between lobes of the lung, within the lobe, or both. Similarly, one might expect the flow of blood within the lungs to be nonuniform, with differences between lobes and/or differences between the individual parts of a single lobe.

The present report is concerned with the differences found in ventilation between and within the lobes of the lung in man and differences which occur within the lobe in ventilation as related to blood flow. Data on ventilation are supplied by continuous nitrogen tracings from the upper and lower lobes while the subject breathes oxygen. Information on ventilation to perfusion relationships is obtained from continuous carbon dioxide tracings from each lobe. The results are qualitative for the present, as differences existing in nitrogen and carbon dioxide concentrations are minimized by mixing within the lobe, and the response time of the two types of meters is so different.

METHODS

The subjects were of both sexes, between the ages of twenty and thirty-five years. All were healthy except for a few tuberculous patients with pneumothorax or pneumoperitoneum who had only minimal disease in the lung at the time of the study. The subjects lay supine on a motor-driven fluoroscopic tilt table. Measurements could then be made with the subject erect or supine without intervening effort on his part.

By a method previously described (2), the upper respiratory tract was anesthetized as for routine bronchoscopy or bronchospirometry. Under fluoroscopic guidance, small, radiopaque, plastic catheters (1 mm. I.D., 2 mm. O.D., and 60 cm. long) were introduced into the right upper and right lower lobe bronchi. Oxygen was supplied through an open system by demand flow. Twin nitrogen meters (Lilly-Hervey type) and twin carbon dioxide meters (3) constantly sampled gas from the right upper and right lower lobe bronchi (figure 1). Pressure gauges in the sampling system were calibrated for flow through the catheters and gave evidence of any obstruction. A flowmeter at mouth level provided expiratory or inspiratory flow rates. When necessary, the catheters were cleared by aspiration. All calibrations were made through dummy catheters similar to those used on the subjects and utilizing gases at body temperature, ambient pressure, saturated (BTPS). The response time of the meters to a sudden change in gas concentration is shown in figure 2. Paper speed in all of the diagrams shown is 25 mm. per second, and time marks are one per second.

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[2] This study was supported, in part, by a grant from the Committee on Medical Research of the American Trudeau Society, medical section of the National Tuberculosis Association; and, in part, by a grant (H-1892) from the Public Health Service.

[3] Presented before the Medical Session, as part of *Section 5B*, at the annual meeting of the National Tuberculosis Association, Milwaukee, Wisconsin, May 25, 1955.

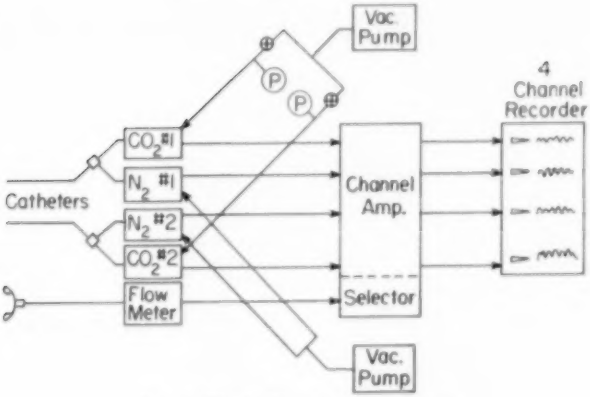

FIG. 1. Block diagram of apparatus.

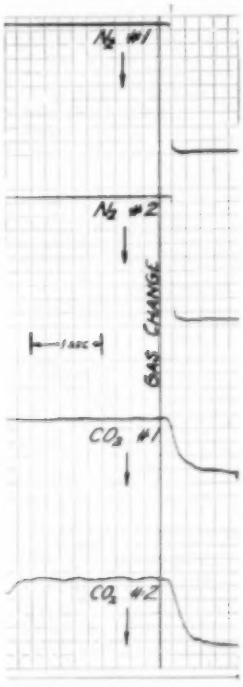

FIG. 2. Response of meters drawing 200 cc. per minute through each catheter.

RESULTS

The expired nitrogen and carbon dioxide plateaus from both lobes in normal subjects showed rising, falling, or constant concentrations (figure 3). These variations were not consistent from breath to breath and were not determined by body

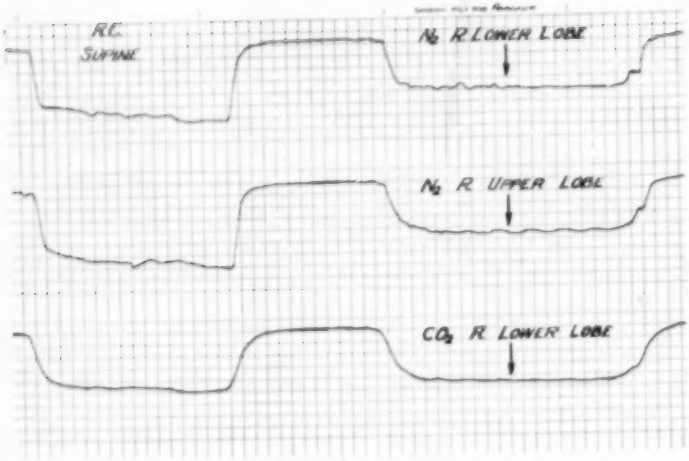

FIG. 3. Record showing nonuniform intralobar ventilation, subject breathing oxygen.

position. Unequal nitrogen concentrations throughout the expiratory plateau were also seen in patients with pneumothorax and pneumoperitoneum.

Nitrogen concentrations changed sharply at the beginning of inspiration in many curves, either rising above or falling below the level at the end of expiration. The inspiratory flow (figure 4) began before this nitrogen change occurred. Frequently, as in figure 4, the direction of these changes was opposite in the upper and in the lower lobe. This characteristic was observed in both the normal subjects and the tuberculous patients.

Nitrogen washout was more rapid in the lower lobe, particularly when the subjects were erect (figure 4). The nitrogen concentration in lower lobe alveolar air was considerably below that in the upper lobe in each breath.

Rapid, brief, nitrogen (figure 5) and carbon dioxide (figure 3) changes synchronous with the arterial pulse occurred during both inspiration and expiration. The carbon dioxide changes were smaller in amplitude, perhaps because of the slow response time of these meters through the catheters. The magnitude of the changes seen during inspiration increased as the flow rate decreased near the end of inspiration. This type of fluctuation was not seen at mouth level. During inspiration, the direction of the changes was the same in both the upper and lower lobes, being always toward the expired concentration; whereas, during expiration, the direction of the changes was unrelated. The expiratory changes of vascular origin often persisted throughout expiration.

Interpretation

In the ensuing discussion the term "uniform ventilation" is used to indicate that during inspiration all alveoli simultaneously receive gas of the same chemical composition and of the same volume in relation to their pre-inspiratory volume (1). Differences in regional ventilation are shown during nitrogen washout when the subject is placed on oxygen. When

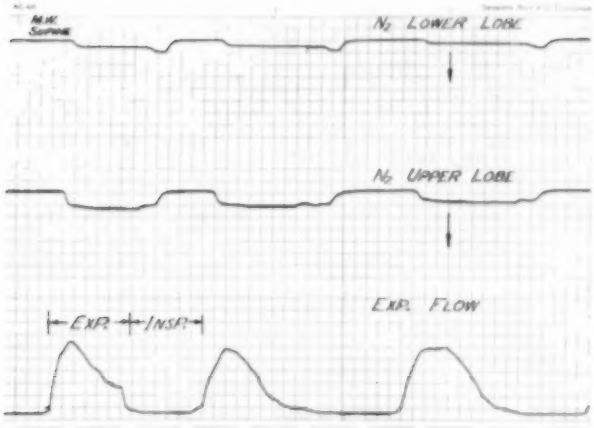

FIG. 4. Record showing dead space inspiration. Subject inspiring oxygen. Time mark at one second. Compared with the upper lobe, the lower lobe N_2 record is delayed 0.12 second.

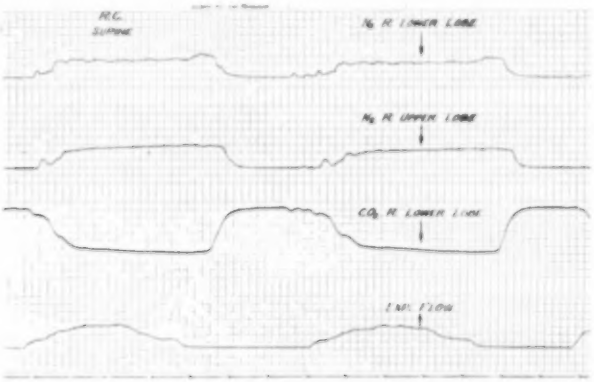

FIG. 5. Record showing concentration changes of vascular origin after nitrogen washout while breathing air. One second time marker is below.

all alveoli within a lobe are uniformly ventilated, the nitrogen concentration will remain constant throughout expiration from that lobe. Concentration variations above or below this value indicate nonuniform ventilation. Gas from poorly ventilated areas will have a relatively high nitrogen concentration and gases from well ventilated areas will have a relatively low nitrogen concentration. Similarly, differences in ventilation between lobes will be shown by a lower mean nitrogen concentration in the better ventilated lobe.

Ventilation was not uniform throughout the individual lung lobe. For example, in the first breath of the tracing which is represented in figure 3, the poorly ventilated areas are apparently emptying late in expiration. While this result was the most common, it was not universal. One breath later there was

less evidence of nonuniform ventilation as the nitrogen concentration was more constant throughout expiration. It is inferred that the nature of intralobar ventilation has changed in both lobes and in the same direction in this particular instance. In some cases, e.g., as in figure 4, nonuniform intralobar ventilation may be more apparent in one lobe than in another.

Nonuniform intralobar ventilation was also revealed by rapid changes in the nitrogen concentration during expiration, which were synchronous with the pulse (figure 5). Presumably these rapid changes reflected regional volume changes of cardiovascular origin. These rapid variations in the expired nitrogen concentration occurred in records from both lobes, but during expiration their directions were unrelated. These nitrogen changes had one or more peaks and their form was usually not as simple as that seen at mouth level.

The fall in nitrogen concentration within the lobe during successive breaths of oxygen is a measure of alveolar ventilation. Because the concentration falls more rapidly in the lower than in the upper lobe, the lower lobe has a relatively greater ventilation. This was characteristic of all of the subjects when studied in the upright position.

In the last third of a second of the nitrogen plateau (figure 4) there was a rise in nitrogen concentration from the lower lobe and a fall in nitrogen concentration from the upper lobe. Flow rates as inscribed below show that these changes occurred during the early part of inspiration. The flowmeter here is on the expiratory side of the open circuit system as this meter was not constructed for use on the inspiratory port. This rise or fall of nitrogen concentration at the beginning of inspiration is interpreted as representing the dead space gas passing the end of the catheters. In this record almost identical concentrations of nitrogen were inspired into both lobes from the dead space. The duration of this dead space gas inspiration was similar for both lobes.

The identification of the dead space gas in this manner provides a graphic demonstration of the difference in ventilation between pulmonary lobes. Ventilation is greater in the lower lobe and the dead space nitrogen concentration inhaled is higher than the concentration which the lobe has just expired. This higher dead space concentration exists because other lung lobes contributing to the dead space are not as well ventilated (*note* upper lobe tracing).

In these records the dead space nitrogen concentration serves as a convenient yardstick for the comparison of interlobar ventilation. Lung lobes with alveolar nitrogen concentrations greater than that present in the dead space are relatively poorly ventilated while those with nitrogen concentrations less than that in the dead space are relatively well ventilated. Thus, on inspection of both figures 4 and 5, the lower lobe is obviously better ventilated than the upper lobe.

The term "uniform perfusion" is used to indicate that at any time in the respiratory cycle all alveoli simultaneously receive gas from the blood of the same chemical composition and at the same rate in relation to their volume. At any time during the expiration those regions having uniform ventilation and uniform perfusion will have the same carbon dioxide concentrations. The concentration itself will be determined by the ratio of ventilation to perfusion. Thus varying carbon dioxide concentrations are evidence of regional differences in the ventilation to perfusion ratio.

In figure 3, changes in carbon dioxide concentration of cardiovascular origin are an index of variation in the ventilation to perfusion ratio within the lobe. These changes cannot be due to a uniform perfusion change since the concentration has a falling phase. It is believed that these changes result from local volume changes causing the preferential emptying of regions within the lobe with varied ventilation to perfusion ratios.

The variation of vascular origin in nitrogen and carbon dioxide concentrations seen during late inspiration (figure 5) were always changes from the inspired level toward the alveolar level of gas concentration. These changes decreased in amplitude as the catheter was withdrawn and increased in amplitude as the flow rates decreased. Early expiratory changes similar to those just described were also seen (figure 5). These changes presumably mean a volume change within the lobe and would suggest that lobar expiration might occur during late inspiration or that lobar inspiration might occur during early expiration.

DISCUSSION

Intralobar ventilation is not uniform. This uneven ventilation is not static, but may change from breath to breath. This implies that the sequence of filling or emptying the lung lobe is not fixed. Factors which might cause this are differences in tidal volume or functional residual volume between breaths, blood volume changes in the thorax causing regional volume changes within the lobe, or variable obstruction to gas flow within the lobe.

Fowler (4) has reported interlobar and intralobar differences in the ventilation of the lower lobes in dogs. He has also suggested (1), as a possible cause of nonuniform ventilation in the lung, the preferential distribution of dead space gas to certain areas. In the breathing depicted in figure 4, dead space gas was drawn into the upper and lower lobes for approximately the same length of time. Furthermore, the concentration of the dead space gas was such that it would have minimized interlobar ventilatory differences. If Fowler's hypothesis is correct and the nonuniformity is due to interlobar differences in distribution, there must have been a relatively much greater volume change in the upper lobe during the period of inspiration of dead space gas. No information on the intralobar distribution of dead space gas is obtainable from these records.[4]

Previously it has been reported from this laboratory (2) that higher alveolar oxygen concentrations are present in the upper lobe than in the lower lobe in the upright subject. This implies that ventilation in relation to perfusion is greater in the upper than in the lower lobe. The present study shows that in the upright subject the lower lobe is better ventilated than the upper lobe. This implies that perfusion per unit of functional reserve volume is considerably less in the upper than in the lower lobe.

Thus the picture presented is one of: (*1*) nonuniformity of ventilation within and between lobes of the lung, (*2*) nonuniformity of ventilation to perfusion

[4] It is apparent that the conditions for calculation of alveolar ventilation at the lobar level are not as simple as those at mouth level since it is necessary to correct for the differences between alveolar concentration and inspired dead space concentrations.

within and between the lobes, and (*3*) nonuniformity of perfusion per unit volume at least between the lobes.

The better ventilation in the lower lobe of erect man described above should provide a higher concentration there of physical and infectious agents carried as gases or droplets. Clinical disease resulting from these agents should occur more often in the lower lobe if concentration alone is considered. Thus inhaled carcinogens should cause more lower lobe than upper lobe carcinoma. The isolated fact of distribution of gases within the lungs may be counteracted by humoral or tissue factors protecting this area.

Dock (5) has postulated that, due to low pulmonary arterial pressures, the upper lobe is poorly perfused with blood in erect man. This will provide higher oxygen tensions (2) in the upper lobe and a better environment for the growth of the tubercle bacillus. This may be a major factor in the localization of adult phthisis to the upper lobe. From the present work it seems that the upper lobe oxygen tension is higher despite better ventilation in the lower lobe of erect man.

SUMMARY

Intralobar ventilation is not uniform in erect or supine man. This nonuniformity is seen in normal subjects and in those with pneumothorax and pneumoperitoneum. It is not consistent and may vary from lobe to lobe and even in the same lobe from breath to breath. Ventilation is better in the lower lobe than in the upper lobe of erect man. The concentration of dead space gas inspired may not be the same as the concentrations existing within the lobe.

The late inspiratory and early expiratory variation in gas concentration within the airways of the lobe provide evidence of regional volume changes resulting from cardiovascular action.

Nonuniform intralobar ventilation to perfusion ratios are seen in normal man. Previous work has shown that the ventilation to perfusion ratio in the lower lobe of erect man is less than that in the upper lobe. This, coupled with the interlobar differences in ventilation shown above, leads to the conclusion that perfusion must be considerably greater in the lower lobe than in the upper lobe, at least in erect man.

SUMARIO

La Ventilación Lobular en el Hombre

La ventilación lobular en el hombre no es uniforme en las posiciones erguida y recostada. Se observa esta falta de uniformidad en los sujetos normales y en los sometidos al neumotórax o al neumoperitoneo. No es constante y puede variar de lóbulo a lóbulo y hasta en cada respiración en un lóbulo. La ventilación es mejor en el lóbulo inferior que en el superior en la posición erguida. La concentración aspirada de gas en los espacios muertos quizás no sea idéntica a las concentraciones que existen dentro de los lóbulos.

La variación aspiratoria tardía y espiratoria incipiente en la concentración de gas dentro de las vías aéreas del lóbulo aporta prueba de que hay alteraciones regionales del volumen debidas a la acción cardiovascular.

En el hombre normal, obsérvanse proporciones disconformes de la ventilación lobular en relación con la perfusión. Estudios anteriores han demostrado que la proporción de la ventilación a la perfusión en el lóbulo inferior del hombre erguido es menor que en el superior.

Esto, unido a las diferencias interlobulares señaladas más arriba en la ventilación, lleva a la conclusión de que la perfusión debe ser considerablemente mayor en el lóbulo inferior que en el superior, por lo menos en la posición erguida.

RESUME

Ventilation lobaire chez l'homme

La ventilation intralobaire n'est pas uniforme chez le sujet couché ou debout. Cette absence d'uniformité se manifeste chez les individus normaux comme chez ceux porteurs d'un pneumothorax ou de pneumopéritoine. Il y a une variation de lobe à lobe et même d'un mouvement respiratoire a l'àutre dans un même lobe. Dans la station debout, la ventilation est meilleure dans le lobe inférieur que dans le lobe supérieur. Dans l'espace mort la concentration de l'air inhalé peut être différente de celle qui existe à l'intérieur du lobe.

La variation de la concentration des gaz dans les voies aériennes du lobe à la fin de l'inspiration et au début de l'expiration démontre qu'il y a des modifications régionales de volume qui sont déterminées par l'action cardio-vasculaire.

La variabilité de la ventilation interlobaire en rapport avec dèbit circulatoire dans le parenchyme se voit chez l'homme normal. Des travaux antérieurs ont montré que le rapport de la ventilation à l'irrigation dans le lobe inférieur, dans la station debout, était inférieur à celui du lobe supérieur. Ce fait, plus les différences interlobaires observées dans la ventilation, permettent de conclure que l'irrigation du parenchyme doit être considerablement plus grande dans le lobe inférieur que dans le lobe supérieur, tout au moins chez l'homme debout.

Acknowledgment

The writers are indebted to Miss Florence Best and Mr. Sergey Politoff for technical help.

REFERENCES

(1) Fowler, W. S.: Intrapulmonary distribution of inspired gas, Physiol. Rev., 1952, *32*, 1.

(2) Martin, C. J., Cline, F., Jr., and Marshall, H.: Lobar alveolar gas concentrations: Effect of body position, J. Clin. Investigation, 1953, *32*, 617.

(3) Young, A. C: Carbon Dioxide Analyzer, Project No. 22-1301-0002, Arctic Aero Medical Laboratory, Ladd Air Force Base, Alaska, 1952, 9 pp.

(4) Fowler, W. S.: Alveolar ventilation of individual pulmonary lobes in various postures, Am. J. M. Sc., 1951, *222*, 602.

(5) Dock, W.: Apical localization of phthisis: Its significance in treatment by prolonged rest in bed, Am. Rev. Tubere., 1946, *53*, 297.

A PSYCHOLOGICAL SCALE FOR PREDICTING IRREGULAR DISCHARGE IN TUBERCULOUS PATIENTS[1]

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(Received for publication May 13, 1955)

INTRODUCTION

In previous publications (1, 2, 3) emphasis has been placed on three aspects of the irregular discharge problem: the need to obtain a better understanding of what impels patients to reject hospitalization and leave prematurely, the need to construct reliable techniques for detecting early in hospitalization those patients who may resort to irregular discharge, and the need to develop workable measures for dissuading such patients from leaving.

The present report is concerned primarily with the second need, i.e., the development of a psychological scale for predicting irregular discharges among tuberculous patients. Secondarily, use of this predictive scale furnishes information relative to understanding the importance of the patient's reactions to tuberculosis and hospitalization as determinants of irregular discharge.

To date, few practical devices have been developed for predicting, on an *individual* basis, whether the tuberculous patient will leave the hospital without medical consent. In a preliminary investigation, it was suggested that certain demographic factors might have value as predictors of future irregular discharge (2). It was found that patients who entered the hospital with far advanced disease, with a history of previous irregular discharge, or who were in the unskilled laborer and unemployed occupational categories were more likely to leave irregularly than patients who did not have these characteristics. In a similar type of study, based on an analysis of admission records, Moran and co-workers (4) also reported the significance of these factors (and others) in discriminating between discharge groups. Such considerations as age, race, religion, marital status, and number of dependents have been found to be of little or no significance as indicators of future irregular discharge. Likewise, the factor of intelligence, as measured by the Wechsler-Bellevue Intelligence Scale, also has been shown to be of negligible value in predicting whether a specific patient will leave the hospital prematurely (5).

Several exploratory attempts to predict irregular discharge by utilizing psychological scales, derived from standard personality tests, have been reported. In an inter-hospital study, in which the application of the Minnesota Multiphasic Personality Inventory (MMPI) to the irregular discharge problem was reviewed, Calden and associates (6) presented a tentative prognostic scale based on MMPI items. Vernier and co-authors (7) also have prepared a tentative predictive index based on patients' responses to the House-Tree-Person projective test.

[1] From the Psychosomatic Department of the Medical and Tuberculosis Service, Veterans Administration Hospital, Madison, Wisconsin.

[2] Deceased.

MATERIALS AND METHODS

The Madison Sentence Completion Form was used in the present study (8). This is a psychological instrument designed specifically to evaluate the attitudes and emotional reactions of tuberculous patients. The form consists of 80 incomplete sentences presented to the patient for completion; for example, "Lying in bed makes me . . . ," "I feel like going home when . . . ," or "What really helps my cure the most is . . . ." By completing these sentences the patient is given an opportunty to express his feelings in regard to bed rest, the hospital, having tuberculosis, the medical staff, the medical treatment, family problems, problems of a general nature, and irregular discharge. The patient's responses to the 80 items, therefore, give a fairly comprehensive picture of how the patient views his hospitalization. This form was utilized because of its general effectiveness in appraising the patient's capacity to adjust to bed rest and hospital living, as well as because of its specific usefulness in offering clues regarding the likelihood that a patient might leave against medical judgment.

During a period of one year, February, 1953, to February, 1954, all male tuberculous patients admitted to the Veterans Administration Hospital, Madison, Wisconsin, were asked to fill out the Madison Sentence Completion Form as part of a routine battery of psychological tests. The form was administered during the second month of hospitalization. In all, 230 patients were tested. This included all patients admitted to the hospital except a small number who were either too ill to be tested or who refused to fill out the form.

In order to develop a scale which would discriminate between discharge groups, it first was necessary to compare the sentence completion responses of the subjects who left irregularly with those who obtained a regular discharge. This was done after a sufficiently large number of patients, constituting a representative sample, had been discharged from the hospital. The unsanctioned discharge subjects consisted of the first 50 patients (32 who left the hospital against medical advice (AMA) and 18 who failed to return from a professionally sanctioned several-day absence from the hospital (AWOL)) in the sample who left the hospital without medical sanction.[1] The sanctioned discharge subjects consisted of the first 50 patients in the sample who obtained a maximum hospital benefit discharge after having been in the hospital for at least three months. At the time of testing, the mean ages of the unsanctioned and sanctioned discharge groups were 38 and 37 years, respectively.

Construction of the Predictive Scale

The predictive scale was constructed in the following manner: Two of the writers, working independently, made a careful analysis and comparison of the sentence completion responses of the two discharge groups. All responses given by the subjects in the sanctioned discharge group to each incomplete sentence were compared with the responses made to the same sentence by the unsanctioned discharge group. The aim of this comparison was to determine whether any given incomplete sentence elicited types of responses from the unsanctioned discharge group which were different from those of the sanctioned discharge group. This procedure was carried out for each of the eighty sentences of the Madison Sentence Completion Form. A sentence was retained for the predictive scale if the responses to it differentiated the two groups at a significant level of 0.20 or less. If the sentence completion responses of the two groups were similar in nature, the sentence was not incorporated into the scale.

When the findings of the two investigators were pooled, it was found that the responses to twenty-two of the eighty sentences were effective discriminators. From this analysis a

[1] Irregular discharge is defined by the Veterans Administration as "any termination of hospitalization of a living patient which is not medically sanctioned by professional authority." There are three categories of irregular discharge: AMA (leaving against medical advice), AWOL (absent without leave), and DD (disciplinary discharge).

Regular discharge in this study applies only to those patients who have completed their hospitalization. Transfers, deaths, and discharges following observation or emergency treatment are not included in the MHB category.

scale was drawn up consisting of these twenty-two sentences and the types of responses which distinguished sanctioned discharge from unsanctioned discharge subjects.

In order to obtain a quantitative measure of "irregular discharge proneness," a simple scoring system was devised as follows: A response was given a score of plus 1 if it reflected the manner in which the unsanctioned discharge subjects completed a sentence, in contrast to that of the sanctioned discharge patients. Similarly, a score of minus 1 was assigned to a response if it had been given more often by the sanctioned discharge subjects. If a type of response did not discriminate between the groups, it was scored 0. A total "predictive" score was computed by algebraically summing up the scores of the responses to the twenty-two significant sentences and adding 50 to this sum. Thus, the predictive score for any given subject could range from 44 to 69. The higher the predictive score, the more likely the patients would be to leave the hospital without medical consent.

In an effort to determine the reliability of the scoring system, each of the 100 test records was scored independently by the two raters according to the guides set forth in the scale. The inter-rater reliability correlation for the two sets of predictive scores was 0.90. This result indicated that there was good agreement between raters. Subsequently, minor changes in the wording of the scale were made as a consequence of scoring discrepancies between raters.

The Madison Irregular Discharge Scale

The scoring manual of the Madison Irregular Discharge Scale is reproduced below. The twenty-two significant sentences are numbered as they occur in the Madison Sentence Completion Form. The designated scores, −1 and +1, are noted beside each response category which discriminates between unsanctioned discharge and sanctioned discharge. Any sentence completion response which does not specifically correspond to the type of examples listed under the sentences in the scale is scored 0.

Madison Irregular Discharge Scale

(Scoring Manual)

2. While I am here . . .
   a. Expresses plans or thoughts along educational or vocational lines.
(−1) "I would like to prepare for entering college."
   "I am thinking things out for some other occupation."
   "I intend to take advantage of Educational Therapy."
   b. Expresses general unhappiness, resentment, weariness, and other complaints.
(+1) "I'm just here, I have to be here"; "nervous."
   "I keep thinking of leaving"; "I wouldn't know."
   "Life is passing me by"; "days seem weeks"; "time drags."
3. When a person is first told he has tuberculosis . . .
   a. Expresses a lack of annoyance or emotional upset. (Do *not* score *disbelief*.)
(+1) "I was surprised"; "reaction varies with the individual."
   "Not too bad, I expected it"; "he wonders to what extent."
   "He shouldn't worry."
6. I feel like going home when . . .
   a. Expresses desire to leave now, constantly, anytime. (Do *not* score responses giving specific reasons, e.g., "the doctors don't tell me about my condition.")
(+1) "Everyday"; "right now"; "in my waking moments."
   "When the idea comes to my head."
   b. Expresses desire to leave when restless, lonely, anxious, "things not right," general discontent. (Do *not* score responses giving specific reasons, e.g., "when I think of home.")
(+1) "I get lonesome and unhappy"; "when I get the blues."
   "I am restless and can't sleep"; "get nervous and upset."

20. My biggest fear . . .
    a. Expresses fear of tuberculosis, fear of non-recovery, relapse, having the disease. (Do *not* score "surgery.")
(−1) "I am not getting well"; "breaking down again"; "death."
    "been handicapped from TB"; "I guess that would be total invalidism."
    b. Denies having fears.
(+1) "No fears"; "is none"; "I ain't scared of nothing."
22. What I like best here is . . .
    a. Expresses no likes—nothing. (Do *not* score "don't know.")
(+1) "I don't care for the hospital."
    "Nothing"; "nothing in particular."
34. The recreation and entertainment . . .
    a. Expresses any unqualified dissatisfaction with recreation and entertainment. (Do *not* score any qualifications, e.g., "is fair, but could be improved.")
(+1) "is poor"; "not enough"; "could be improved"; "more needed on wards"; "where is it?"
36. A patient feels like walking out of here . . .
    a. Expresses desire to leave when despondent, moody, depressed, restless, lonesome. (Do *not* score responses giving specific reasons, "when I don't get a pass.")
(+1) "When he is at his lowest morale"; "during moods."
    "When he gets to worrying"; "when he's down in the dumps."
    b. Expresses desire to leave because of home, family, loneliness.
(+1) "When he wants to be with his family."
    "When things go wrong at home"; "when he is lonesome."
40. I worry most about . . .
    a. Expresses chief or first worry in relation to family or home.
(+1) "My wife and mother"; "my children"; "my family and my lungs."
    "Personal problems about my family at home."
42. This hospital is like . . .
    a. Expresses thought that hospital is usual or commonplace, same as others.
(+1) "A hospital"; "most TB hospitals"; "many others."
    "The other hospital I was in"; "all hospitals are the same."
43. Maybe I wouldn't have gotten sick if . . .
    a. Indicates that drinking or "dissipating" may have been a causative factor of his illness.
(+1) "I found different work and quit the booze"; "I drank with TB guys and didn't know it"; "dissipating in bars"; "I had not got drunk every day."
44. When a patient moves to another ward . . .
    a. Indicates this is a sign of medical improvement or advance in treatment.
(−1) "He'd be doing OK—improving"; "it's a step forward."
    "He'd know he's on his way out."
46. I expect to stay here until . . .
    a. Indicates that the patient will leave *only* with the staff's permission—"when told to." (Do *not* score "cured," or "I get well.")
(−1) "They tell me"; "doctor OK's it"; "I am told I can go."
    "I am discharged"; "the hospital releases me."
50. Filling out a form like this . . .
    a. Expresses resistance and negativism to filling out the form.
(+1) "Is confusing to me."
    "Seems like nonsense."
    "Is to see how nuts I am"; "won't do anyone any good."
51. The future looks . . .
    a. Expresses pessimism. (Do *not* score "uncertain," "questionable.")
(+1) "Cloudy and cooler"; "gloomy"; "pretty bad right now."
    "Not so hot"; "rough."

52. The patients' main gripes . . .
    a. Complains about visitors or pass situation.
(+1) "The visiting hours are at a bad time"; "not enough passes."
    "Not enough visitors"; "I want a pass."
53. Having TB wouldn't be so bad if . . .
    a. Indicates that patient's spreading it to others, contagion, is worst aspect.
(−1) "It wasn't contagious"; "you couldn't give it to anyone."
    "It wouldn't be endangering other people."
    b. Indicates doubt as to cure or permanency of cure.
(−1) "It could be cured instead of arrested"; "no breakdown."
    "Medical world knew more about it"; "the bug could be killed."
    "I am cured."
    c. Indicated desire for outpatient treatment, treatment at home.
(+1) "It could be cured at home."
    "One could stay at home and be with one's family."
    "It could be handled through outpatient treatment."
    d. Unrealistically denies illness.
(+1) "You didn't have it"; "a person didn't have TB."
59. A real friend . . .
    a. Expresses lack of friendship or cynicism about friendship.
(+1) "Hard to find"; "is me"; "there are none"; "there are few."
    "I don't know"; "is a dollar bill."
60. This sentence completion form . . .
    a. Indicates form is definitely *foolish*, or *silly*, or waste of time. (Do *not* score lack of understanding of purpose of test.)
(+1) "Seems silly to me but guess it must be for a reason."
    "Is foolish"; "is a big joke."
66. The length of time I plan to stay in the hospital . . .
    a. Indicates that he will leave *only* when allowed or cured.
(−1) "Until I'm discharged"; "when they tell me I can go."
    "Up to doctors"; "whatever time is necessary"; "until I'm cured."
71. My plans for the future . . .
    a. Expresses uncertainty or lack of plans. (Do *not* score any qualifications, e.g., "depends on my case.")
(+1) "Uncertain"; "?"; "undetermined"; "rather indefinite."
    "I have no plans"; "I don't know yet."
73. What I like best when I'm sick . . .
    a. Desires to be left alone, unmolested, asocial. (Do *not* score "peace and quiet," "quiet.")
(+1) "Is to be left alone"; "is solitude"; "not to be bothered."
78. I would feel better if . . .
    a. Expresses desire to go home, or see more of family.
(+1) "I were home with my family"; "I could see more of my wife and family"; "I could go home for Christmas."
    "My wife could visit me every day."

RESULTS

Validation of Predictive Scale

When applied to the original criterion groups, i.e., the sample of 50 unsanctioned discharge and 50 sanctioned discharge patients, the scale effectively discriminated between discharge groups at a high level of significance ($p < 0.001$). The mean score of the unsanctioned discharge group was 53.72 and

TABLE 1

EFFECTIVENESS OF MADISON IRREGULAR DISCHARGE SCALE SIGN SCORES WHEN APPLIED TO THE ORIGINAL SAMPLE OF 100 PATIENTS

Predicted Discharge Status	Sign Score	Actual Discharge Status		Total
		MHB*	ID†	
Unsanctioned discharge	53 and over	6	35 (91%)	41
"?"	51–52	12	12	24
Sanctioned discharge	50 and under	32 (85%)	3	35
	Total	50	50	100

* Sanctioned discharge
† Unsanctioned discharge

the mean score of the sanctioned discharge group was 50.06. Inspection of the distribution of scores revealed that the scale was maximally effective if a sign score of 53 and above was used to characterize "irregular discharge proneness," and a sign score of 50 or less was indicative of "maximal hospital benefit discharge proneness." An intermediate score of 51 or 52 did not enable one to place the subject clearly in a "proneness" category. Applying this cutting score technique to the distribution of unsanctioned discharge scores indicated that it was possible to have made discharge predictions in 76 per cent of the cases. For the predicted cases, the correct percentage of predicted unsanctioned discharge patients was 85 per cent. The correct percentage of predicted sanctioned discharge patients was 91 per cent. The discriminative efficiency of the scale for the criterion groups is presented in table 1.

These preliminary results seemed very promising. However, it was necessary to cross-validate the scale on an independent sample before it could be established that this procedure was a valid screening device for predicting unsanctioned discharge. The scale, therefore, was applied to new samples of 25 unsanctioned discharge and 25 sanctioned discharge patients whose discharges occurred *after* those of the first group of patients. This validation sample was selected by essentially the same criteria as was the original sample. The mean age of the 25 unsanctioned discharge patients was forty-one years at the time of testing. The mean age of the 25 sanctioned discharge patients was forty-two years.

The results of the application of the predictive scale and the cutting points to the new group are shown in table 2. If each of these 50 test records had been scored immediately after they had been filled out, the scale would have predicted the type of discharge with an efficiency approximately equal to that of the original sample. Definite predictions could have been made in 60 per cent of the cases. For these cases, the correct percentage of patients predicted "irregular discharge" was 88 per cent. The correct percentage of predicted "maximal hospital benefit" cases was 83 per cent. The scale effectively discriminated between the discharge groups of the validation sample at a high level of significance ($p < 0.001$). In this cross-validation the mean predictive score of the

TABLE 2

EFFECTIVENESS OF MADISON IRREGULAR DISCHARGE SCALE SIGN SCORES WHEN APPLIED TO THE CROSS-VALIDATION SAMPLE OF 50 PATIENTS

Predicted Discharge Status	Sign Score	Actual Discharge Status		Total
		MHB*	ID†	
Unsanctioned discharge	53 and over	2	16 (88%)	18
"?"	51–52	13	7	20
Sanctioned discharge	50 and under	10 (83%)	2	12
	Total	25	25	50

* Sanctioned discharge

† Unsanctioned discharge

unsanctioned discharge group was 53.28 and the mean score of the sanctioned discharge group was 50.44.

As a consequence of the above results on both the original and validation groups, it is believed that the scale will predict with sufficient accuracy to warrant its continued use for individual prediction of irregular discharge.

DISCUSSION

The Madison Irregular Discharge Scale was designed primarily as a practical device for detecting, early in hospitalization, those patients who were likely to leave irregularly. However, in addition to its usefulness in prediction, the responses on the scale furnished many specific insights into the relationship of the patient's attitudes and unsanctioned discharge. The list of sentence completion responses given more frequently by these patients can be grouped into definite patterns which reflect attitudes to their illness, their hospitalization, and to factors outside the hospital. These are clearly different from those expressed by the sanctioned discharge patients.

It is generally assumed that all tuberculosis patients are highly fearful of their disease and are strongly motivated toward cure. However, an analysis of the responses given by unsanctioned discharge and sanctioned discharge patients to the sentences of the scale shows that this assumption is not entirely true. In general, patients who subsequently obtain irregular discharges are less able or willing to accept their illness and their treatment program. The unsanctioned discharge patients are more inclined to underestimate, show less concern for, and (in some cases) even deny the existence of their disease. Their response to tuberculosis is characterized by *under-reactivity*. For example, when asked to express their first reaction to the diagnosis of tuberculosis (sentence 3), a number of unsanctioned discharge patients shrugged their shoulders and responded in a relatively unemotional fashion. In contrast to the sanctioned discharge patients, who responded with such characteristic expressions as "I was shocked," "It was a low blow," "It threw me for a loop," the unsanctioned discharge patients were inclined to complete the sentence with such toned-down attitudes

as "It was not too bad, I expected it," "You shouldn't worry," or "There are worse sicknesses."[4]

Similarly, when asked to indicate their biggest fear (sentence 20), the unsanctioned discharge patients more readily denied having fears. "I ain't scared of nothing." The sanctioned discharge patients, however, usually told about their fears and apprehensions concerning their illness. Their most common fears were related to the possibility of not recovering, of suffering a relapse, or of being physically handicapped. The subjects in the sanctioned discharge group also appeared more upset over the fact that they might infect others (sentence 53).

Despite these expressed fears about their illness, the sanctioned discharge patients seemed more optimistic about the future (sentence 51) and showed a more positive orientation in terms of recovery (sentence 44). Also of interest to investigators, who found a linkage between alcoholism and unsanctioned discharge, were the comments of several unsanctioned discharge patients concerning their drinking problems (sentence 43). They tended to attribute the illness to "too much boozing." Such responses were rarely given by the sanctioned discharge patients.

As was anticipated, the unsanctioned discharge patients revealed a negative attitude toward many phases of their hospitalization. They complained about being unhappy, about being bored or depressed, or about feeling weary while in the hospital (sentence 2). "Time drags," "Life is passing me by," "I get nervous around here," "I sure am down in the dumps" were common complaints. Criticisms against the recreational and entertainment facilities of the hospital also occurred more frequently.

Many of the unsanctioned discharge patients found it difficult to establish personal ties and friendships during their hospital stay. They did not perceive the hospital as a substitute "home away from home," but rather as just another impersonal institution (sentence 42). This attitude was reflected in such statements as "this hospital is like all the others" and "All hospitals are the same. If you've been in one, you've been in all." This unwillingness to personalize their hospital stay was also seen in their negativistic responses to the two sentences that permit them to express their views about the hospital (sentences 22 and 42). Either the unsanctioned discharge subjects stated that they "don't care for nothing here" or they implied, in a rather perverse, asocial fashion, that they liked being here mainly because the hospital offered them an opportunity to hibernate away from personal contacts. For example, they would claim that what they liked best was "not to be bothered by people" or "to be left alone." This desire to live in an isolated social vacuum and to avoid close

[4] It may be contended that the attitudes of under-reactivity toward disease may, in part, be a function of extent of disease. However, an analysis of admission records showed that the unsanctioned discharge patients of the present study had a disproportionately greater percentage of far advanced disease in contrast to the patients who obtained sanctioned discharges. In other words, the unsanctioned discharge patients were more inclined to deny or underevaluate their illness *despite* the fact that they were more seriously ill.

contact with others in the hospital was also reflected in their cynicism about friendship (sentence 59). For example, the sentence "a real friend . . ." is often completed by the unsanctioned discharge patients with the phrase "there is no such thing" or "a five dollar bill."

Related to these expressions of social isolation and general negativism was a marked resistance among some unsanctioned discharge patients to filling out the sentence completion form (sentences 50 and 60). They tended to suspect the motives behind the distribution of the form or regarded the filling out of the form as "a total waste of time" or as "just a lot of nonsense, to see how nuts I am."

In contrast to the predominantly negativistic attitudes evinced by the unsanctioned discharge patients, the sanctioned discharge group seemed more positively oriented toward their hospitalization. They viewed the many months spent in the hospital as more than a mere empty passage of time. They thought that their hospital time could be used to advantage (sentence 2) by taking educational courses, by preparing for a new vocation, or turning toward other constructive activities.

In contrast to the lesser degree of positive hospital orientation revealed by the unsanctioned discharge patients is their greater concern about problems *outside* the hospital. Separation from family and difficulties at home appeared more pressing to the unsanctioned discharge patients than to the sanctioned discharge group. As shown in the scale (sentence 40), they were more inclined to worry about "personal problems about my family at home." They repeatedly expressed a yearning to be home with the family, rather than remain in the hospital (sentence 78). While they were in the hospital, they also were more prone to complain about the pass situation or about any restrictions placed on visitors (sentence 52). Coupled with this outside-the-hospital orientation was a strong preference for obtaining their medical treatment at home, on an outpatient basis (sentence 53).

It is difficult to say whether the family problems of unsanctioned discharge patients were objectively more difficult than those encountered by the sanctioned discharge patients. However, they did perceive their problems as being more burdensome, stressful, and anxiety provoking. It is interesting to note that their expressed worry, loneliness, and dependency on those outside the hospital contrasted strongly with the inability of many unsanctioned discharge patients to form friendships and emotional attachments *within* the hospital (sentences 59 and 73).

The responses to four of the incomplete sentences dealing with premature discharge likewise revealed differences in attitudes between the two groups. Leaving the hospital against the doctor's wishes was more often contemplated by the unsanctioned discharge patients, and was more readily viewed as a solution to their feelings of discontent engendered by hospitalization. The unsanctioned discharge patients stated (sentences 6 and 36) that they felt like going home, or felt like "walking out of here" "when I get the blues," "during moods," "when I get restless," and "whenever the idea comes to my head." Loneliness,

the desire to be with one's family, and problems at home are listed by these subjects as prominent factors prompting the patient to leave irregularly.

The attitudes of the sanctioned discharge patients toward irregular discharge, on the other hand, were consistent with their more positive orientation toward their illness, their treatment, and their hospitalization. They asserted that they planned to remain in the hospital until they were cured and were discharged with the staff's consent. For example (sentence 46), they maintained that they expected to stay until "the doctor OK's it" or "I am told to go by the hospital authorities." Similarly, they felt that the length of time they planned to stay in the hospital was "up to the doctors" and "when they tell me I'm cured."

An analysis of the attitudes expressed by the unsanctioned discharge patients suggests that there are certain characteristic personality patterns which seem linked with irregular discharge.

In a previous publication (1), a provisional collection of personality features of unsanctioned discharge patients was presented, based mainly on clinical observations. Prominent among these character traits is the inability of these patients to establish close emotional attachments to people or situations within the hospital. For example, it was pointed out that there are many of the unsanctioned discharge patients who experience marked "separation anxiety" and are unable to tolerate removal from loved ones who remain outside the hospital. Such "home-centered" persons have "fixed dependencies" upon wife, parent, or job, and apparently are incapable of transferring these dependency relationships to anyone within the hospital. These are the patients who, on the Madison Sentence Completion Form, indicate how strongly they miss their families or who worry excessively over family problems. Their responses also indicate a readiness to leave the hospital whenever they feel lonely or depressed or whenever restrictions are placed upon visiting hours.

A second group of unsanctioned discharge patients, who seem incapable of developing close attachments in the hospital, fall more into the "social isolate" category. These individuals characteristically wander into the hospital as strangers and wander out as strangers, thereby repeating a life pattern of withdrawal from close personal contacts whether within or outside a hospital setting. In filling out the form, they are most likely to say that "being left alone" is what they like best in the hospital. They are unable to perceive the hospital as a particular place with specific assets or disadvantages, but tend to lump it together with all other hospitals. "It's like all the other hospitals." They are also inclined to deny the seriousness of their illness and express little confidence in their own capacity to get well. There is a general lack of hope and a depressive tone to all of their activities. A number of these patients may appropriately be diagnosed as having "schizoid" personalities.

A third group consists of unsanctioned discharge patients who show strong ambivalences in their attachments to staff personnel, fellow patients, and hospital routines. During their hospital stay, they alternately feel fondness and dislike for the people about them, and "love turns to hate" whenever there is frustration of their desires. Many in this group are "emotionally trigger happy," i.e.,

they have low tolerance for frustration and react in an exaggerated way to relatively minor deprivations. Their cry, "Give me my clothes!" is usually uttered in the heat of anger. These "over-reactors" indicate in their sentence completion responses that they are perpetually restless and discontented and that they are liable to leave the hospital at a moment's notice whenever things go wrong.

By contrast, sanctioned discharge patients are more likely to be mature enough to confront their disease realistically and face the frustrations inherent in the hospital treatment. Despite the impact of tuberculosis upon their lives, with its attendant disruptions of normal living, they feel hopeful about their recovery and can plan for the future. Many of them have sufficiently developed emotional controls to permit them to utilize educational therapy and other productive channels for satisfying their needs and for preserving emotional stability while adhering to the treatment program. In addition, most patients in this group show sufficient social maturity to care about other people and to consider the danger of infecting them if they leave the hospital prematurely (in contrast to the self-centered attitude of many unsanctioned discharge patients). Of particular importance is their capacity to develop confidence in the hospital personnel. This is evidenced, on the form, in the sanctioned discharge patients' expressed trust in their doctors and nurses and their unwillingness to leave the hospital unless it meets with the approval of the medical staff.

As indicated above, there is considerable agreement between the earlier clinical observations and the findings obtained on the predictive scale. The attitudes and personality traits exhibited by unsanctioned discharge patients are probably not restricted to the hospital setting alone, but are aspects of long-standing patterns of behavior. These patterns appear characteristic of early infancy and childhood and reveal a stage of personality development which, in psychoanalytic terms, may be described as "oral narcissism." The responses given on the form show marked congruence with attitudes associated with this period of personality development. In essence, the data indicate that most unsanctioned discharge patients have immature personalities reflecting disturbances in very early interpersonal relationships.

SUMMARY

The aim of this study was to devise a psychological scale to aid in predicting, early in hospitalization, the likelihood of unsanctioned discharge of tuberculous patients. This was done by comparing the responses to the Madison Sentence Completion Form given by patients who left the hospital without medical consent (unsanctioned discharge) with those given by patients who obtained a maximal-hospital-benefit type of discharge (sanctioned discharge). The resulting Madison Irregular Discharge Scale consists of twenty-two incomplete sentences which elicit responses that effectively discriminate between groups of discharge patients. The scale not only discriminated between the original criterion groups with a high degree of accuracy, but worked effectively on independent, cross-validation groups.

The attitudes toward tuberculosis and hospitalization, embodied in the predictive scale, reflect a number of significant differences in attitudes between discharge groups. In contrast to those who obtain a maximal-hospital-benefit type of discharge, patients who leave without medical sanction indicate early in hospitalization that they are more inclined to underestimate both the seriousness of their disease and the necessity for treatment, are more pessimistic about the future, reveal a more negative attitude toward hospitalization, have difficulty in establishing friendships in the hospital, and are more disturbed by family and social problems outside the hospital. Unsanctioned discharge patients, on the whole, appear to be more immature than patients who are discharged with medical consent.

SUMARIO

Escala Psicológica para Predecir el Alta Irregular de los Tuberculosos

Tuvo por propósito este estudio idear una escala psicológica que ayudara a predecir, a principios de la hospitalización, las probabilidades de alta irregular en los tuberculosos. Se hizo esto comparando las respuestas al Formulario Mádison para Completar Oraciones, dadas por enfermos que abandonaron el hospital sin consentimiento médico con las dadas por enfermos que obtuvieron un alta basada en el máximo beneficio hospitalario posible. La resultante Escala Mádison de Alta Irregular consta de 22 oraciones incompletas que reclaman respuestas que diferencian positivamente distintos grupos de enfermos dados de alta. La escala no sólo diferenció con mucha exactitud entre los primitivos grupos tomados como normas sino que también funcionó eficazmente en grupos independientes de valores cruzados.

Las actitudes hacia la tuberculosis y la hospitalización, encarnadas en la escala de predicción, reflejan varias diferencias importantes en la actitud de los grupos dados de alta. En contraposición a los que obtienen un alta basada en el máximo beneficio hospitalario posible, los enfermos que abandonan el hospital sin sanción médica indican a principios de la hospitalización que: se muestran más inclinados a menospreciar tanto la gravedad de su enfermedad como la necesidad de tratamiento, son más pesimisitas acerca del futuro, revelan una actitud más negativa hacia la hospitalización, encuentran más dificultad para hacer amistades en el hospital, y se hallan más perturbados por problemas familiares y sociales fuera del hospital. En conjunto, los enfermos de alta irregular parecen ser más inmaduros que los dados de alta con consentimiento médico.

RESUME

Une échelle psychologique de prédiction du départ non autorisé des tuberculeux hospitalisés

L'objectif de cette étude était l'élaboration d'une échelle psychologique pour faciliter la prédiction, au début de l'hospitalisation, de la possibilité du départ non autorisé du malade tuberculeux. Ceci a été effectué en comparant les réponses au questionnaire du test de Madison (Sentence Completion Form) obtenues chez les patients qui ont quitté l'hôpital sans autorisation médicale avec celles données par les malades qui ont obtenu le résultat maximum du séjour à l'hôpital. L'échelle "Madison Irregular Discharge Scale" obtenue consiste en 22 phrases incomplètes provoquant des réponses permettant d'établir une discrimination entre les groupes de malades "sortants". Cette échelle non seulement permet une discrimination entre les groupes critères originaux avec un haut degré de précision, mais elle s'est également appliquée efficacement aux groupes indépendants, qui ont permis de mettre à l'epreuve la valeur de l'échelle établie.

Les attitudes envers la tuberculose et l'hospitalisation exposées dans l'échelle de prediction, reflètent un certain nombre de différences significatives parmi ces attitudes entre

les groupes de "sortants". A l'encontre de ceux qui obtiennent le profit maximum du séjour à l'hôpital, les malades qui sortent sans autorisation médicale indiquent dès le début de l'hospitalisation: qu'ils sont plus disposés à sous-estimer à la fois la gravité de leur maladie et la nécessité du traitement, qu'ils sont plus pessimistes quant à l'avenir, qu'ils ont une attitude plus négative vis à vis de l'hospitalisation, qu'ils éprouvent une difficulté à établir des relations d'amitié à l'hôpital, qu'ils sont plus troublés par les problèmes familiaux et sociaux de l'extérieur. Les malades appartenant au groupe des départs non autorisés apparaissent plus dépourvus de maturité que les malades dont le départ de l'hôpital est sanctionné médicalement.

REFERENCES

(1) Lewis, W. C., Lorenz, T. H., and Calden, G.: Irregular discharge among tuberculosis patients: A major unsolved problem, Psychosom. Med., 1955, *17*, 276.

(2) Lorenz, T. H., Lewis, W. C., Calden, G., and Thurston, J. R.: An investigation of irregular discharge in tuberculous patients: Report of a preliminary study, Am. Rev. Tuberc., 1955, *71*, 419.

(3) Lorenz, T. H., Green, J. M., Lewis, W. C., Stone, M., Calden, G., and Thurston, J. R.: Investigation of irregular discharge of tuberculosis patients: A special ward procedure for reducing AMA discharges, Am. Rev. Tuberc., 1955, *72*, 633.

(4) Moran, L. J., Fairweather, G. W., Morton, R. B., and McGaughran, L. S.: The use of demographic characteristics in predicting response to hospitalization for tuberculosis, J. Consult. Psychol., 1955, *19*, 65.

(5) Thurston, J. R., and Calden, G.: Intelligence factors in irregular discharge among tuberculosis patients, J. Consult. Psychol., 1954, *13*, 404.

(6) Calden, G., Thurston, J. R., Stewart, B. M., and Vineberg, S. E.: The use of the MMPI in predicting irregular discharge amoung tuberculosis patients, J. Clin Psychol., 1955, *11*, 374.

(7) Vernier, C. M., Whiting, J. F., and Meltzer, M. L.: Index for prediction of AMA discharge for TB patients, reported in Newsletter for Psychologists in Tuberculosis, 1954, *2*, 8.

(8) Calden, G.: A method for evaluating the attitudes of tuberculous patients, Am. Rev. Tuberc., 1953, *67*, 722.

THE CLINICAL INCIDENCE OF ACID-FAST BACILLI NONPATHOGENIC FOR GUINEA PIGS[1]

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(Received for publication August 11, 1955)

INTRODUCTION

After Koch (1) isolated the tubercle bacillus in 1882 and proved its pathogenicity, Theobald Smith (2) established the pathogenic nature of human and bovine tubercle bacilli for both man and cattle. Since that time various related acid-fast bacilli have been discovered which are nonpathogenic for man. Among these are the avian tubercle bacilli, smegma bacilli, and various saprophytic bacteria found in the soil and in and upon animals.

One generally accepted test of the pathogenicity of acid-fast bacilli is their ability to produce and be recovered from tuberculous lesions in guinea pigs, although this may cover only one aspect of pathogenicity. In 1918, Corper (3) reported that acid-fast bacilli isolated from the sputum of two patients failed to produce typical tuberculosis in guinea pigs. Subsequently a number of investigators have reported more than 120 cases in which nonpathogenic acid-fast bacilli were isolated from various human sources (3–15). Atypical chromogenic colonies developed in cultures from some of these patients (4, 7, 10, 12, 14); however, some chromogenic acid-fast bacilli may have varied pathogenicity for guinea pigs (14). Steinberg, Holzberger, and Schwartz (13) described 17 patients with acid-fast bacilli in the sputum, but with normal chest roentgenograms. Twelve other patients had pulmonary infiltrations of apparently nontuberculous nature. Other writers have reported similar cases (4, 5, 7, 10).

Several investigators have described the isolation of acid-fast bacilli which were avirulent for guinea pigs from patients with typical clinical evidence of tuberculosis (8, 12, 14, 15).

The possible connection of antituberçulous chemotherapy with the increasing incidence of nonpathogenic acid-fast bacilli has been postulated (14–16). In a few cases it has been suspected that atypical mycobacteria, which produced nontuberculous lesions or no pathology in guinea pigs, may have been responsible for heretofore unrecognized disease entities in human beings (5, 7, 9, 11).

Most of the previous case reports have indicated only scattered and occasional instances of the isolation of guinea pig avirulent acid-fast bacilli from human sources. The following cases are reported because of the increasingly frequent occurrence of such findings in a wide variety of case material.

METHODS AND MATERIALS

During the years 1953 and 1954, 46 cases were collected at the Uncas-on-Thames Sanatorium of patients from whom acid-fast bacilli which were nonpathogenic for guinea pigs had been isolated. The patients were selected from both the outpatient and inpatient serv-

[1] From the Uncas-on-Thames Sanatorium, Norwich, Connecticut.

ices, which numbered about 1,900 and 500 annual visits and admissions, respectively, during these two years.

Sputum studies (or gastric aspirates) were done on all inpatients, but only on those outpatients who had expectoration. Sputum (or urine in one case) was digested and concentrated by adding an equal amount of 4 per cent sodium hydroxide. The concentrate was homogenized for ten minutes with a paint shaker, incubated at 37°C. for one hour, centrifuged for fifteen minutes, titrated to pH 6.8, and then spread on slides and inoculated onto tubes of culture medium.

A modified American Trudeau Society medium (potato, glycerine, egg yolk, and 2 per cent malachite green) was used. The cultures were incubated aerobically at 37°C. for eight weeks.

Colonies were considered to be typical of tubercle bacilli if they grew fairly slowly, were friable and adherent to the medium, and were cream- or slightly buff-colored. The cultures were not routinely examined for cord formation. Cultures composed of markedly chromogenic colonies and those with unusually shaped acid-fast bacilli or organisms which did not stain uniformly were considered to be composed of atypical acid-fast bacilli.

For positive cultures, whether typical or atypical, guinea pig inoculation was the test of pathogenicity employed. Cultures were sent to the laboratories of the Connecticut State Department of Health at Hartford. There, smears were made to confirm the presence of acid-fast bacilli, after which 2 guinea pigs were inoculated as follows:

> A loopful of culture material was homogenized and suspended in one ml. of normal saline and then injected into the right groin of the guinea pig. The guinea pigs were promptly autopsied at death or at the time of sacrifice eight weeks later. The organisms were not considered to be tubercle bacilli unless "acid-fast organisms were removed from lesions typical of tuberculous infection."

RESULTS

The 46 cases were arbitrarily divided into five major groups (table 1). In all cases one or more cultures of sputum concentrates or urine (one case only) were positive for acid-fast bacilli which were nonpathogenic for guinea pigs.

Normal Pulmonary Parenchyma on Chest Films

Seven patients had nonpathogenic acid-fast bacilli in the sputum with normal appearing pulmonary parenchyma on roentgenograms of the chest. Three of the 7 patients had prominent left hilar markings; 2 had calcified left hilar lymph

TABLE 1

CATEGORIES OF FORTY-SIX PATIENTS FROM WHOM ACID-FAST BACILLI LACKING PATHOGENICITY FOR GUINEA PIGS WERE ISOLATED

Category		Number of Patients
Normal pulmonary parenchyma on chest films		7
Abnormal, probably nontuberculous lung lesions on chest films		7
Tuberculous lung lesions on chest films of patients with probably inactive disease		30
No previous antituberculous chemotherapy	12	
With previous antituberculous chemotherapy	18	
Tuberculous lung lesions on chest films of patients with probably active disease		1
Probably active urinary tract tuberculosis		1

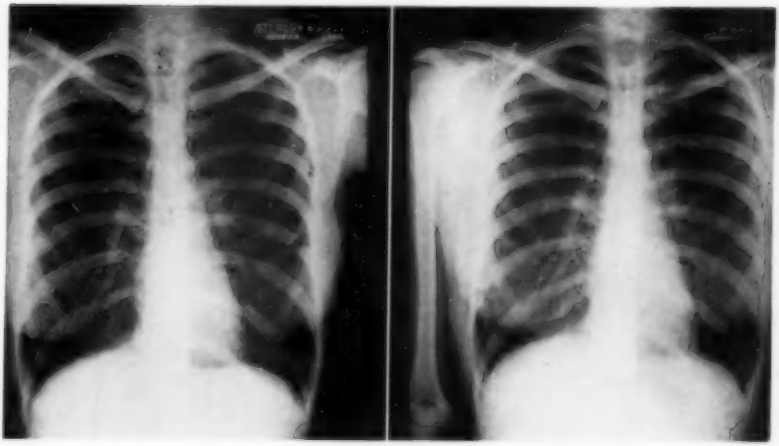

FIG. 1. *Patient* R. D. H. These normal chest roentgenograms were taken in March and August, 1954. Acid-fast bacilli which were nonpathogenic for guinea pigs were cultured from the sputum in May, 1954.

nodes; 2 had entirely normal chest films. All 7 patients were reactors to tuberculin. Their main symptom was cough; 2 had hemoptysis. Two to eight sputum studies were done on each patient. All preliminary smears of sputum concentrates were negative for acid-fast bacilli. Seven cultures found to be nonpathogenic on guinea pig inoculation had been considered to show growth typical of tubercle bacilli.

The age of the patients varied from five to sixty-six years. Five were males and 2 were females. A wide variety of occupations was represented. All patients were residents of eastern Connecticut.

Case history: R. D. H. was a 32-year-old white housewife with a one-year history of productive cough, posterior chest pain, night sweats, and slight bloody streaking of the sputum. She had had rheumatic fever as a child without rheumatic heart disease, pneumonia in 1948, and pleurisy in 1951. Physical examination was essentially normal. Six seventy-two-hour sputum concentrates in April and May, 1954, were negative for acid-fast bacilli on microscopy. Four cultures were negative; one produced atypical, chromogenic acid-fast bacilli; and a second culture typical of tubercle bacilli was nonpathogenic on guinea pig inoculation. The chest films were normal (figure 1). The patient reacted to second-strength PPD. Subsequent extensive examination at the Hartford Hospital, which included sputum studies, bronchoscopy, chest films, and bronchograms, indicated no pulmonary disease.

Abnormal, Probably Nontuberculous Lung Lesions on Chest Films

Seven patients had abnormal pulmonary lesions which were probably nontuberculous. The chief symptom in 6 patients was productive cough; 3 patients had hemoptysis; 2 had chest pain. On the basis of serial chest roentgenograms, sputum studies, and other diagnostic procedures, the final diagnoses in these 7 patients were: nontuberculous bronchiectasis (by bronchograms); cystic

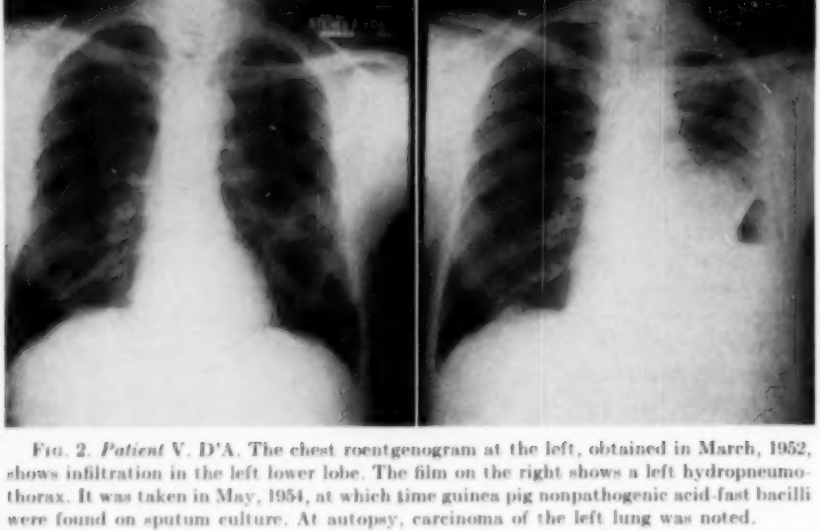

FIG. 2. *Patient* V. D'A. The chest roentgenogram at the left, obtained in March, 1952, shows infiltration in the left lower lobe. The film on the right shows a left hydropneumothorax. It was taken in May, 1954, at which time guinea pig nonpathogenic acid-fast bacilli were found on sputum culture. At autopsy, carcinoma of the left lung was noted.

disease of the lungs (by laminagrams); interstitial fibrosis probably due to silicosis; 2 cases of nontuberculous pneumonia; and 2 cases of carcinoma of the lung. In one of the last-named patients a nontuberculous empyema and pericarditis were present and there was a minute fibrocaseous nodule of questionably tuberculous nature in the periphery of the noncancerous lung.

Six patients were positive reactors to tuberculin. The seventh patient had failed to react to first strength PPD, but the second-strength PPD was not used. Three to eighteen sputum studies were done on each patient. All smears of sputum concentrates, with one exception, were negative for acid-fast bacilli. Of seven culture strains which were nonpathogenic for guinea pigs, only one was described as chromogenic. The organisms in another culture did not stain uniformly; five cultures were reported as containing acid-fast bacilli typical of tubercle bacilli.

The patients' ages varied from fifty to seventy-two years. Only one patient was a female. One patient was a stonecutter; the remainder had various other occupations. All resided in eastern Connecticut.

Case history: V. D'A. was a 58-year-old Italian bartender who was first seen in 1952 with a diagnosis of left lower lobe pneumonitis of unknown etiology (figure 2). The patient had a six-month history of productive cough, night sweats, and dyspnea. Bronchoscopy was normal and five specimens of sputum were negative for acid-fast bacilli. He was readmitted in 1954 with left chest pain, cough, night sweats, bloody streaking of the sputum, and weight loss. The patient had smoked one package of cigarettes per day for forty-six years. Physical examination revealed signs of fluid in the left lower chest. Chest films showed a left hydropneumothorax and a questionable mass along the left cardiac border (figure 2). The cutaneous reaction to second-strength PPD was positive. Nine sputum

concentrates were negative for acid-fast bacilli on microscopy. Only one culture was positive and was typical of tubercle bacilli; however, it proved to be nonpathogenic on guinea pig inoculation. Shortly after admission, thoracotomy drainage of a left empyema was performed. Pneumococci, but no acid-fast bacilli, were found in the pus from the empyema. Bronchoscopy subsequently showed stenosis of the left lower lobe bronchus and cytologic examination of the washings was positive for tumor cells. Thoracotomy revealed inoperable carcinoma of the left lung. Examination of material obtained by pleural biopsy indicated epidermoid carcinoma. The patient died one month later, and autopsy revealed carcinoma of the left lung with pleural extension. There was no evidence of tuberculosis in the lungs.

Pulmonary Tuberculosis Demonstrable Roentgenographically but Presumably Inactive

No Previous Antituberculous Chemotherapy

There were 12 patients in the group with inactive pulmonary tuberculosis who had received no antituberculous chemotherapy. Nine of these patients had been followed for three to twenty-two years; 3, for only one year. In 1953 and 1954 all patients had clinical and roentgenographic evidence of minimal to far advanced, fibrotic or fibrocalcific pulmonary tuberculosis, which probably was inactive. Nevertheless, 2 patients still had evidence of persisting cavitation. Six patients had had classical symptoms of tuberculosis in the past and had been hospitalized in the sanatorium for active tuberculosis. Five had no symptoms or only mild coryza, and the lesions were discovered on routine chest films. One

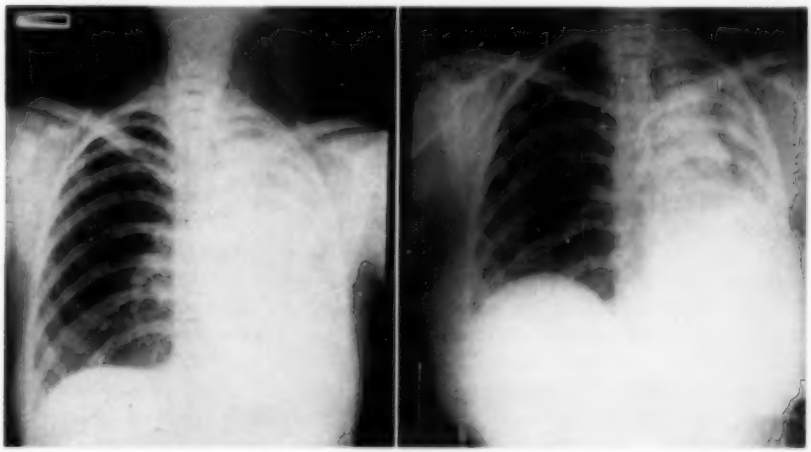

FIG. 3. *Patient* A. K. R. The chest roentgenogram at the left, obtained in March, 1942, shows far advanced tuberculosis involving primarily the left lung. The patient had five sanatorium admissions between 1935 and 1947, with active tuberculosis, after which time her disease seemed stable and inactive. The film on the right was taken in June, 1954, at which time acid-fast bacilli which produced no tuberculosis on guinea pig inoculation were found in the sputum. She had received no antituberculous chemotherapy.

patient had severe pulmonary emphysema, as well as evidence of old fibrocalcific tuberculosis.

Eight patients had positive reactions to tuberculin; the remaining 4 were not tested. Practically all of the smears and cultures of sputum concentrates from these patients were negative for acid-fast bacilli between 1951 or earlier and the present study in 1953 and 1954. Two of the 13 cultures of acid-fast bacilli which produced no tuberculosis in guinea pigs were described as chromogenic; one as otherwise atypical; 10 were typical of *M. tuberculosis*. All of these sputum specimens were negative for acid-fast bacilli on microscopic examination.

The ages of the patients ranged from twenty-eight to seventy-two years. The 5 women were all housewives. Three of the 7 men were chicken farmers or farm hands; one had been a stonecutter. All resided in eastern Connecticut.

Case history: A. K. R. was a 48-year-old white housewife. She had developed pulmonary tuberculosis in 1932 and had been admitted to the sanatorium five times between 1935 and 1947 because of hemoptyses and reactivation of her tuberculosis. Her sputum was intermittently positive for acid-fast bacilli on microscopy and culture during these years. Chest films showed far advanced pulmonary tuberculosis, with minimal infiltration at the right apex, and extensive fibrocavitary disease with fluid and pleural thickening on the left (figure 3). After 1947, yearly sputum studies were negative for tubercle bacilli except for one positive culture in 1951 and one in 1954. The former was not subjected to a test of virulence; the latter, composed of apparently typical tubercle bacilli, was nonpathogenic for guinea pigs. During this period the patient was relatively asymptomatic, and roentgenographic abnormalities were stable. At no time had the patient received antituberculous chemotherapy.

Inactive Tuberculosis with Previous Chemotherapy

Eighteen patients with probably inactive pulmonary tuberculosis had received antituberculous drugs before acid-fast bacilli, with no pathogenicity for guinea pigs, were isolated from the sputum. Fifteen patients previously had had classical symptoms of tuberculosis, with bacteriologic and roentgenographic evidence of moderately to far advanced pulmonary tuberculosis. Three still had evidence of possible cavitation at the time of this study. Two patients also had diabetes mellitus, 2 probably had silicosis, and one also had cystic bronchiectasis.

All patients had received streptomycin or dihydrostreptomycin and sodium para-aminosalicylate (PAS) before the atypical acid-fast bacilli were cultured from the sputum, and all but 4 had received at least one year of therapy with these drugs. Four patients also had received isoniazid for one to nine months. Nine patients were still receiving chemotherapy at the time of the positive culture; 9 had had no chemotherapy for one to six years. Eight patients also had had previous surgery. Included in the surgical procedures were two lobectomies with segmental resections and space-filling thoracoplasties, four segmental resections (one with thoracoplasty and paraffin plombage on the opposite side), and two three-stage thoracoplasties (one with paraffin plombage on the opposite side).

Twelve patients had positive tuberculin reactions. In 6, the tuberculin reactions were not reported. Each of the patients had long series of sputum studies.

Sixteen had had negative cultures for more than one year at the time of the positive cultures under study. A series of sputum concentrates, which yielded 21 cultures of acid-fast bacilli without pathogenicity for guinea pigs, was positive for acid-fast bacilli on microscopy in only two instances. Six of these cultures were chromogenic; two were otherwise atypical; thirteen cultures appeared to be composed of typical tubercle bacilli.

One patient had received streptomycin-PAS for ten months and then had a segmental resection of part of the diseased lung tissue. One year after completion of chemotherapy, a chromogenic acid-fast bacillus was cultured from the sputum and the organisms were nonpathogenic for guinea pigs. One year thereafter the patient's sputum contained typical tubercle bacilli which were pathogenic for guinea pigs, although clinically and roentgenographically the patient showed no evidence of active disease.

The ages of the patients varied from sixteen to seventy-three years at the time of the study. There were 8 female and 10 male patients. Five of the women were housewives; one male was a stonecutter, and another a farmer. The occupations of the others varied. Their residences were scattered widely over Connecticut.

Case history: T. K. S. was a 38-year-old white housewife who had had an infiltration in the right lung since 1943. She was admitted to the sanatorium in 1952 after six weeks of cough, one episode of questionable hemoptysis, and a weight loss of 8 pounds. Physical examination was unremarkable. Chest films showed moderately advanced pulmonary tuberculosis with cavitation in the right upper and lower lobes (figure 4). The skin reaction

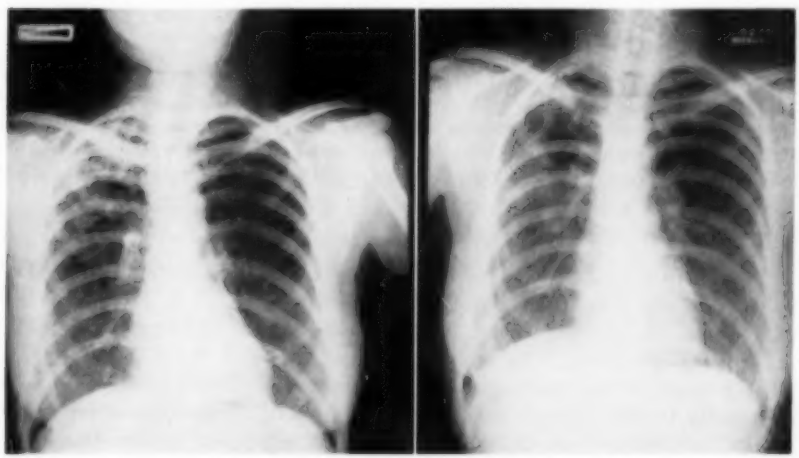

FIG. 4. *Patient* T. K. S. The chest roentgenogram on the left, obtained in March, 1952, shows moderately advanced pulmonary tuberculosis. Subsequently the patient had one year of streptomycin-PAS and segmental resections in the right lung, with apparent arrest of her disease. The film on the right was taken in April, 1954, at the time of a mild pneumonitis of the right lung which rapidly cleared on treatment with chlortetracycline. At this time acid-fast bacilli, which lacked pathogenicity for guinea pigs, were found in one sputum culture.

to the second-strength PPD was strongly positive. Several smears and cultures of sputum were positive for acid-fast bacilli. The patient had received streptomycin-PAS for eight months; then resection of the apical and posterior segments of the right upper lobe and superior segment of the right lower lobe was carried out. The resected lung tissue contained caseous foci of tuberculosis, and bronchiectatic changes were present in the tissue from the right upper lobe. Smears of the tissue were positive for acid-fast bacilli, but no growth was obtained on cultures. Postoperatively the patient had therapy for four more months with streptomycin-PAS. During the following year she remained well. Sixteen sputum cultures were negative for acid-fast bacilli. One culture was subsequently obtained which was typical of *M. tuberculosis* morphologically but failed to produce disease in guinea pigs. This was at a time when the patient had a brief episode of "viral" pneumonia in the right lung, which cleared rapidly on chlortetracycline therapy.

Pulmonary Tuberculosis Demonstrable Roentgenographically and Presumably Active Disease

A 66-year-old white male tobacco farmer had had far advanced bilateral pulmonary disease for at least three years, with typical symptoms of tuberculosis. Chest roentgenograms showed far advanced disease with a giant cavity in the right upper lobe. Despite a long course of streptomycin, dihydrostreptomycin, isoniazid, and PAS, the sputum was consistently positive for acid-fast bacilli by smear and culture, and the patient's general condition continued to deteriorate. One of the thirty positive cultures was composed of acid-fast bacilli morphologically typical of *M. tuberculosis* but nonpathogenic for guinea pigs. Two months later chromogenic organisms were grown from the sputum. In subsequent months, acid-fast bacilli, morphologically typical of tubercle bacilli, were isolated but were not tested in guinea pigs.

Probably Active Urinary Tract Tuberculosis

A 40-year-old white male metal finisher, with moderately advanced pulmonary tuberculosis, previous diagnoses of tuberculosis of the urinary tract and probably the spine, and Hodgkin's disease, received streptomycin-PAS for thirteen months in 1952 and 1953. The sputum became negative for acid-fast bacilli early in 1953. In February, 1954, a twenty-four-hour voided urine was positive on culture for acid-fast bacilli which were avirulent for guinea pigs. Two months later urine cultures from both ureters yielded acid-fast bacilli which were pathogenic for guinea pigs. Isoniazid-PAS therapy was started, but the patient left the hospital against medical advice.

DISCUSSION

This study emphasizes one of the pitfalls in the diagnosis of active tuberculosis, namely that the demonstration of acid-fast bacilli in the sputum by either smear or culture does not absolutely establish the identity of the disease or its state of activity. The morphologic appearance of the cultures is not a reliable index of their pathogenicity for guinea pigs. Thirty-seven cultures of apparently typical tubercle bacilli were found to be nonpathogenic for guinea pigs. Although nine chromogenic and four otherwise atypical cultures from these patients proved to be nonpathogenic for guinea pigs, there is evidence that such microorganisms may occasionally display some pathogenicity (14).

The results of pathogenicity studies in guinea pigs provide no certain information concerning pathogenicity for other animal species. What is needed is a simple, rapid, accurate test of virulence for cultures and, even better, for organisms found on smears. Cytochemical tests may offer some promise for future

realization of this goal. In the meantime, the over-all clinical picture must be relied upon heavily in the diagnosis of pulmonary tuberculosis and in determinations of its activity.

In the differential diagnosis of other chest conditions it is also becoming evident that acid-fast bacilli may be found in the sputum of apparently nontuberculous patients. The 14 nontuberculous patients in the present study who produced sputum with acid-fast bacilli which were nonpathogenic for guinea pigs serve to emphasize this point.

Several investigators have suggested that antituberculous chemotherapy encourages the development of avirulent acid-fast bacilli (14–16). Forty-six cases are an insufficient number from which to draw accurate conclusions. Nevertheless, the fact that 26 of the 46 patients had received no previous drug therapy may indicate that other factors are also at work in the finding of acid-fast bacilli which are nonpathogenic for guinea pigs. Further, it is interesting that 9 of the 20 patients with previous drug therapy had not received drugs for a year or more at the time of isolation of the nonpathogenic acid-fast bacilli.

SUMMARY

Forty-six patients were seen at the Uncas-on-Thames Sanatorium during the years 1953 and 1954. These patients were excreting acid-fast bacilli which were nonpathogenic for guinea pigs. Seven of these patients had normal chest films and 7 had nontuberculous lung disease. Thirty patients had probably inactive pulmonary tuberculosis, 18 of whom had received previous antituberculous chemotherapy; one patient had probably active pulmonary tuberculosis; and one had probably active urinary tract tuberculosis. Fifty cultures of acid-fast bacilli obtained from these patients failed to produce tuberculosis in guinea pigs. Of these cultures, thirty-seven were described as composed of typical growths of tubercle bacilli, nine were chromogenic, and four showed other atypical characteristics.

The occasional finding of acid-fast bacilli which were nonpathogenic for guinea pigs in the sputum of tuberculous and nontuberculous patients emphasizes one major pitfall in the diagnosis of active tuberculosis and the differential diagnosis of other chest diseases. Antituberculous drugs may play a role in developing avirulent strains of tubercle bacilli. Nevertheless, acid-fast bacilli avirulent for guinea pigs, but morphologically identical with virulent tubercle bacilli, may be found in patients with or without previous demonstrable tuberculosis who have not received chemotherapy.

SUMARIO

La Incidencia Clínica de Bacilos Acidorresistentes No Patógenos para el Cobayo

Durante los años 1953 y 1954 se observó en el Sanatorio Uncas-on-Thames a 46 enfermos de los cuales se aislaron bacilos ácidorresistentes que resultaron avirulentos para los cobayos. Siete de estos enfermos mostraron parénquima pulmonar normal en las radiografías torácicas; 7 tenían afección pulmonar no tuberculosa; 30 tenían tuberculosis pulmonar probablemente inactiva, habiendo recibido 18 de ellos quimioterapia antituberculosa previa-

mente; un enfermo tenía tuberculosis pulmonar probablemente activa; y uno tenía tuberculosis probablemente activa del aparato urinario. Cincuenta cultivos obtenidos de estos enfermos que resultaron positivos para bacilos tuberculosos no produjeron tuberculosis en cobayos. De estos cultivos, a 37 se les describió como compuestos de típicas colonias de bacilos ácidorresistentes, 9 eran cromógenos y 4 fueron clasificados como atípicos.

El hallazgo de vez en cuando de bacilos ácidorresistentes que no eran patógenos para los cobayos en el esputo de enfermos tuberculosos y no tuberculosos recalca un escollo importante en el diagnóstico de la tuberculosis activa y en el diagnóstico diferencial de otras afecciones torácicas. Las drogas antituberculosas pueden desempeñar un papel en la producción de cepas avirulentas de bacilos tuberculosos; no obstante, en enfermos con o sin tuberculosis descubrible anteriormente que no han recibido fármacoterapia, pueden descubrirse bacilos ácidorresistentes avirulentos para el cobayo, que parecen idénticos culturalmente a los bacilos tuberculosos virulentos.

RESUME

La fréquence clinique de bacilles acido-résistants non pathogènes pour le cobaye

L'isolement de bacilles acido-résistants avirulents pour le cobaye a été effectué chez 46 malades du sanatorium de Uncas-on-Thames entre 1953 et 1954. Les clichés du poumon ont montré un parenchyme normal dans 7 cas; une affection non tuberculeuse dans 7 cas; la tuberculose stabilisée était probable dans 30 cas. Parmi ceux-ci 18 malades avaient été antérieurement soumis à la chimiothérapie: 1 malade paraissait atteint de tuberculose pulmonaire en activité; chez 1 malade la tuberculose des voies urinaire paraissait probable. Cinquante cultures positives pour le BK provenant de ces malades, n'ont pas tuberculisé les cobayes. Parmi ces cultures, 37 sont décrites comme étant composées de colonies de bacilles acido-résistants, neuf d'entre elles étaient chromogènes et quatre atypiques.

La découverte occasionnelle de bacilles acido-résistants non pathogènes pour le cobaye dans l'expectoration de malades tuberculeux, ou non tuberculeux, attire l'attention sur un important traquenard du diagnostic de la tuberculose en activité et du diagnostic différentiel dans d'autres affections du poumon. Il est possible que les antibiotiques jouent un rôle dans l'apparition de souches avirulentes du bacille tuberculeux; néanmoins, des bacilles acido-résistants non virulents, dont l'aspect en culture est identique à celui des bacilles virulents, peuvent être découverts chez des sujets avec ou sans atteinte antérieure de tuberculose apparente et n'ayant pas été soumis à la chimiothérapie.

REFERENCES

(1) Koch, R.: Die Aetiologie der Tuberculose, Berl. klin. Wchnschr., 1882, *19*, 221.

(2) Smith, T.: A comparative study of bovine tubercle bacilli and of human bacilli from sputum, J. Exper. Med., 1898, *3*, 451.

(3) Corper, H. J.: The virulence of the tubercle bacilli isolated from the sputum, J. Infect. Dis., 1918, *23*, 493.

(4) Baldwin, E. R.: Nonpathogenic acid-fast bacilli: I. A case of chronic pneumonitis associated with a nonpathogenic acid-fast bacillus, Am. Rev. Tuberc., 1942, *45*, 756.

(5) Beaven, P. W., and Bayne-Jones, S.: Mycobacterium (sp.?), Ryan strain, isolated from pleural exudate, J. Infect. Dis., 1931, *49*, 399.

(6) Branch, A.: A study of acid-fast organisms other than mammalian tubercle bacilli isolated from disease in man, Tubercle, 1933, *14*, 337.

(7) Buhler, V. B., and Pollak, A.: Human infection with atypical acid-fast organisms: Report of two cases with pathologic findings, Am. J. Clin. Path., 1953, *23*, 363.

(8) Corper, H. J., and Cohn, M. L.: The presence of relatively avirulent tubercle bacilli in pulmonary tuberculosis in man, J. A. M. A., 1942, *120*, 427.

(9) Cummins, S. L., and Williams, E. W.: An "acid-fast" other than the Koch's bacillus cultivated from sputum, Tubercle, 1933, *15*, 49.

(10) Feldman, W. H., Davies, R., Moses, H. E., and Andberg, W.: An unusual mycobacterium, isolated from sputum of a man suffering from pulmonary disease of long duration, Am. Rev. Tuberc., 1943, *48*, 82.

(11) Hauduroy, P.: Inventaire et Description des Bacilles Paratuberculeux, Masson et Cie, Paris, 1946.

(12) Pinner, M.: Atypical acid-fast microorganisms. III. Chromogenic acid-fast bacilli from human beings, Am. Rev. Tuberc., 1935, *32*, 424.

(13) Steinberg, D., Holzberger, P. C., and Schwartz, G.: False positive bacteriological reports in diseases simulating pulmonary tuberculosis, Dis. of Chest, 1951, *20*, 277.

(14) Tarshis, M. S., and Frisch, A. W.: Chromogenic acid-fast bacilli from human sources, Am. Rev. Tuberc., 1952, *65*, 278, 289.

(15) Yegian, D., and Budd, V.: Certain nonpathogenic mycobacteria, Am. Rev. Tuberc., 1953, *68*, 557.

(16) Morse, W. C., Weiser, O. L., Kuhns, D. M., Fusillo, M., Dail, M. C., and Evans, J. A.: Study of the virulence of isoniazid-resistant tubercle bacilli in guinea pigs and mice, Am. Rev. Tuberc., 1954, *69*, 464.

THE PATHOGENESIS OF TUBERCULOSIS AS SHOWN IN STUDIES OF OMENTAL SPREADS[1]

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(Received for publication August 10, 1955)

INTRODUCTION

This report is concerned with a study of the changes which occur in the omentum of guinea pigs and rabbits from the first hour until thirty days after intraperitoneal inoculation. In most of the animals this inoculation was a primary infection; in the others it was a reinfection following an earlier intramuscular inoculation into the thigh.

The two aims of the experiment were: first, to observe the differences in host-parasite relationship when diverse mycobacteria were introduced; and, second, to study more closely the changes which lead up to necrosis and caseation.

Woodruff (1) has already published an account of the growth of tubercle bacilli in the omenta of normal and allergic guinea pigs. Gardner (2, 3) described the tissue reactions to primary infection and reinfection with tubercle bacilli and made the important observation that, in the peritoneal cavity, hypersensitivity is initiated by the sudden reversal of the monocyte-lymphocyte ratio and is characterized by a high relative and absolute lymphocytosis. There are good reasons, some of which are presented by Woodruff, Kelly, and Leaming (4), for believing that conditions in the infected omentum simulate the naturally infected lung more closely than those in the infected skin or eye.

Although the results of the present study are in some measure a confirmation of the bacteriologic and histologic observations of Woodruff and Gardner, respectively, the conclusions drawn go further and are at variance with Gardner on the relationship of lymphocytosis to immunity.

MATERIAL AND METHODS

Guinea pigs weighing between 200 and 300 gm. and rabbits weighing between one and two kg. were used. To study the omental reaction in sensitized animals, prior inoculation into the thigh with a living culture of H37Rv was made two to three weeks before intraperitoneal inoculation, and sensitization was confirmed by the tuberculin reaction.

The strains of mycobacteria used were H37Rv, H37Ra, BCG, *M. phlei*, *M. smegmatis*, and a saprophytic strain recovered from tap-water. All strains were grown in Glover's medium: for ten days in the case of H37Rv, H37Ra, and BCG, and for two days for the saprophytic strains. They were washed and resuspended in a concentration of 1 mg. wet weight per ml. of fresh Glover's medium and were inoculated on the basis of 0.1 mg. per 100 gm. weight of animal.

Ten cubic centimeters of air were inoculated intraperitoneally to raise the peritoneum, immediately before the inoculation of the bacilli, and the inoculation was made in the midline midway between the xiphoid process and the symphysis pubis. After appropriate intervals the animals were killed by chloroform and the omentum was excised and spread directly

[1] From the Institute of Orthopaedics and Royal National Orthopaedic Hospital, London, W. 1, England.

onto slides. It was sometimes necessary to float it out in saline before spreading. After drying at 37°C., the omenta were fixed in methyl alcohol and stained with Giemsa, hematoxylin and eosin, or Ziehl-Neelsen stains or by auramine-rhodamine. Large omenta were divided and the portions stained by different methods; small omenta were stained by rhodamine-auramine first and restained for histology after localizing the bacilli.

The position and arrangement of bacilli were determined by fluorescent microscopy as well as by examination of the Ziehl-Neelsen preparations by a ×45 fluorite objective. The cytology of the omenta was studied in hematoxylin-eosin and Giemsa-stained preparations, but all of the photographs are of Ziehl-Neelsen-stained preparations; the Giemsa stain diffused out of the cells so rapidly that it was not possible to keep them long enough for color photography, which could be done only once a week, and the hematoxylin-eosin stain did not provide sufficient color contrast.

Two hundred and thirty-two guinea pigs were used for repeating Woodruff's experiments; 13 were given nitrogen mustard in an attempt to produce lymphopenia. The nitrogen mustard CB 1348[2] was suspended in arachis oil and given subcutaneously; 12.5 mg. per kg. was the dose used for the initial inoculations; subsequent doses were varied according to the weight and leukocyte response.

OBSERVATIONS

Omental Changes in Guinea Pigs Following Intraperitoneal Introduction of M. Tuberculosis Hominis (H37Rv)

Unsensitized guinea pigs: The sequence of changes in the omentum when tubercle bacilli of the H37Rv strain were introduced into the peritoneal cavity of the normal guinea pig are presented chronologically.

At one hour there was a phagocytosis of the bacilli by "free" monocytes and eosinophils.

At three hours those monocytes which had ingested bacilli had agglutinated in clumps on the omental surface; such clumps apparently form the core of future tubercles. Neutrophils migrating from capillaries had begun to phagocytose bacilli and to agglutinate (figure 1). Cords of bacilli appear to attract neutrophils rather than monocytes.

At one day inflammatory changes had appeared and vast numbers of neutrophils had migrated from the capillaries. Macrophages in the perivascular areolar tissue and in "milk spots" were proliferating; many bacilli found in these areas had apparently stuck on the surface.

At three days the inflammatory reaction was subsiding. Neutrophils were still clustering around cords of bacilli. The monocyte clumps were growing and attracting neutrophils (figure 2).

At four days the bacilli were multiplying extracellularly on omental cells away from tubercles. The inflammatory reaction had subsided.

By the fourth to ninth day the tubercles had increased in size and density. There was an increase of neutrophils on the omental surface and in the peritoneal exudate. The macrophages of the milk spots and perivascular connective tissue had continued to proliferate.

By the tenth to twentieth day there were typical tubercles composed of epithelioid

[2] Kindly supplied by Dr. L. A. Elson of the Chester Beatty Research Institute, London, England.

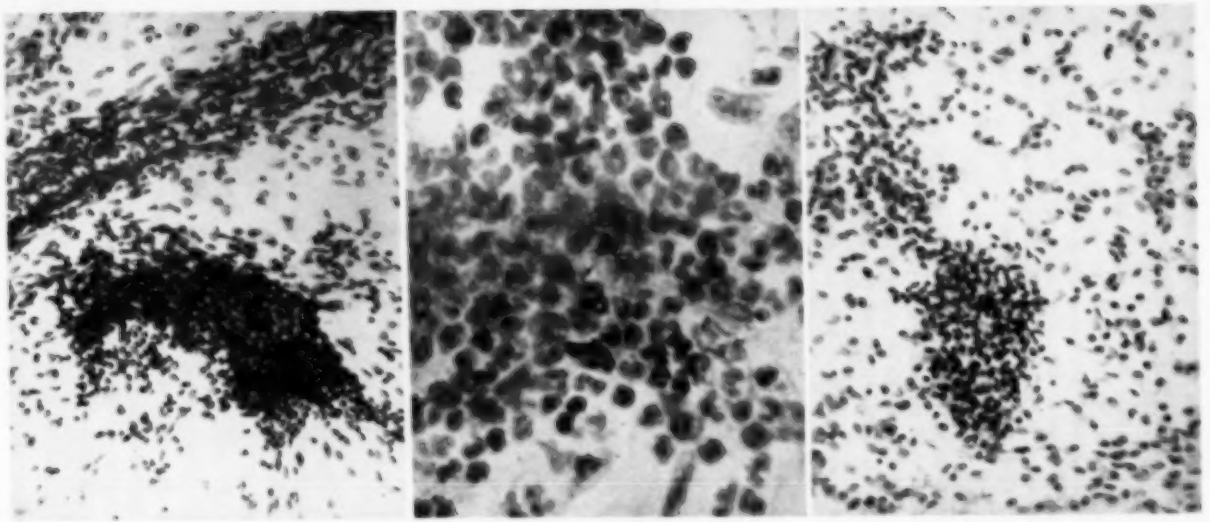

FIG. 1 FIG. 2 FIG. 3

FIG. 1. Guinea pig omentum three hours after inoculation with H37Rv. Neutrophils moving out of capillary toward a clump of monocytes.

FIG. 2. Guinea pig omentum three days after inoculation with H37Rv. Early tubercles, composed of monocytes and neutrophils. A cluster of tubercle bacilli in center.

FIG. 3. Guinea pig omentum ten days after inoculation with H37Rv. Lymphocytes appeared for first time.

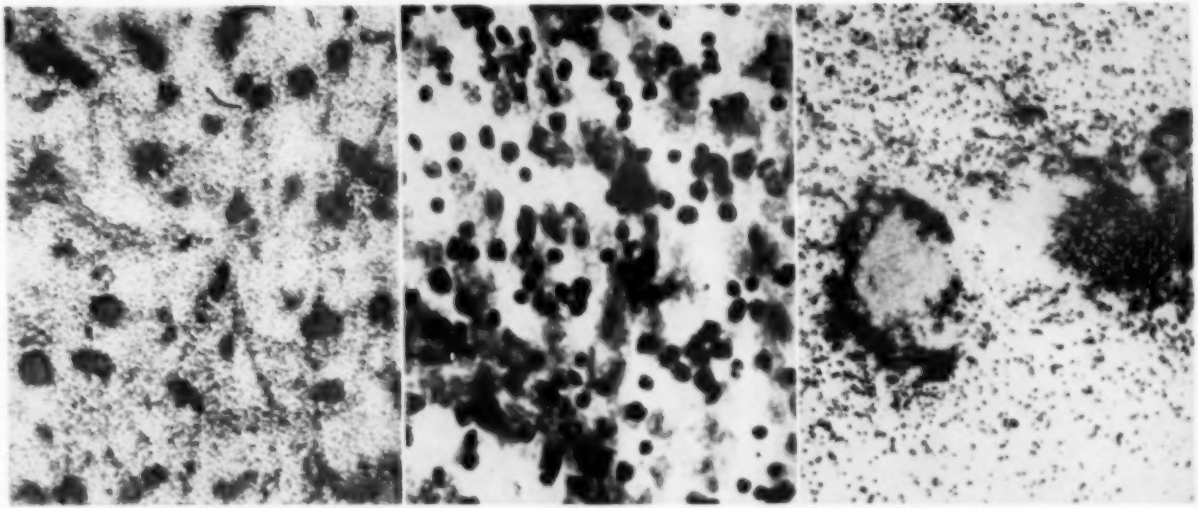

FIG. 4 FIG. 5 FIG. 6

FIG. 4. Guinea pig omentum nineteen days after inoculation with H37Rv. Low-power picture of nodular tubercles scattered over omentum.

FIG. 5. Guinea pig omentum twenty-eight days after inoculation with H37Rv. High-power picture of lymphocytes at the edge of a tubercle.

FIG. 6. Guinea pig omentum thirty-two days after inoculation with H37Rv. Low-power picture of caseous areas with lymphocytes.

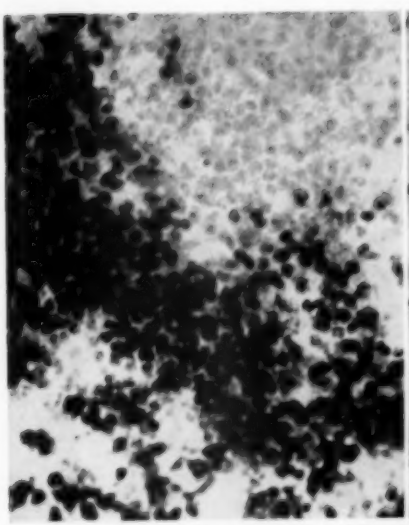

FIG. 7

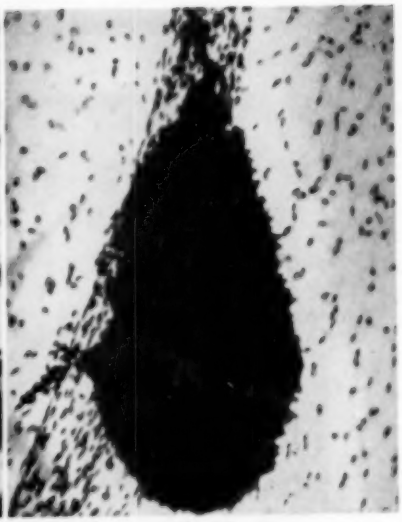

FIG. 8

FIG. 7. Guinea pig omentum thirty-two days after inoculation with H37Rv. High-power picture of lymphocytes at margin of caseous area.

FIG. 8. "Sensitized" guinea pig omentum one day after inoculation with H37Rv. Dense clump of monocytes beside a capillary.

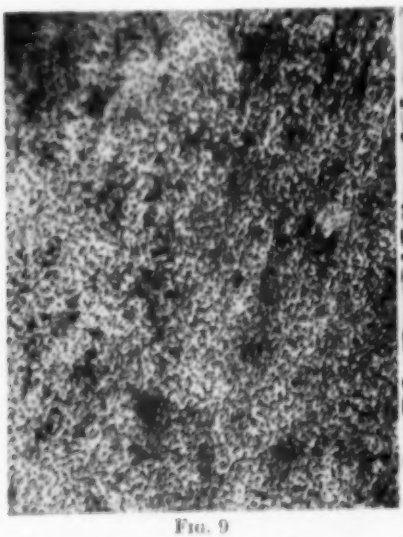

FIG. 9

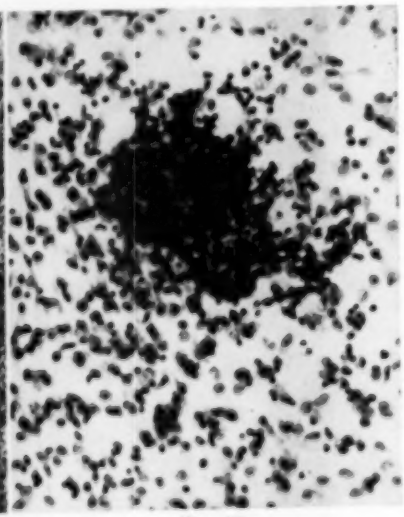

FIG. 10

FIG. 9. "Sensitized" guinea pig omentum four days after inoculation with H37Rv. Low-power picture of omentum showing a cellular activity not found before the third week in the unsensitized.

FIG. 10. "Sensitized" guinea pig omentum four days after inoculation with H37Rv. Lymphocytes around a tubercle.

cells of loose structure containing large numbers of bacilli that appeared to be dividing. Lymphocytes which had hitherto been rare were now apparently increasing and beginning to surround and invade the tubercles. *By the nineteenth day*, necrosis had occurred in the center of many tubercles (figures 3, 4).

By the twenty-first to twenty-eighth day the invasion of the tubercles by lymphocytes had led to greatly increased density. Bacillary multiplication had apparently decreased and the bacilli were reduced in numbers. Macrophages, histiocytes, and neutrophils were also present, but there were no giant cells (figure 5).

From the fifth week onwards the focal necrosis had become generalized caseation (figures 6, 7). The lymphocytes, which had invaded the tubercles, and the epithelioid cells had lost their outlines, and their nuclei had become fragmentary. Bacilli embedded in this caseous material were sometimes abnormally long but most were shorter than normal. *By the seventh week* the fibrosis was extensive in certain areas.

Guinea pigs previously sensitized with H37Rv: At three hours there appeared to be monocytes which formed larger clusters and ingested more bacilli than had been observed in the normal animal infected with H37Rv by the intraperitoneal route.

By one to six days a progressive proliferation of monocytes had occurred (figure 8), but the most striking feature was the early appearance and rapid increase in lymphocytes. The peritoneal exudate showed a reversal of the monocyte-lymphocyte ratio from the first day. Neutrophils were almost absent and there was no inflammatory rise as seen in the normal pig. The bacilli appeared to have been rapidly enveloped in sheets of cells and their multiplication was not obvious (figures 9, 10).

At nine days focal necrosis was now evident in many tubercles. After nine days the omenta of sensitized guinea pigs became so thick and adherent to adjacent viscera that it was impossible to spread them satisfactorily for study.

Omental Changes Following Intraperitoneal Introduction of Other Mycobacteria

The sequence of changes in the omentum when other strains of mycobacteria were introduced into the peritoneal cavity is presented in summary form.

M. Tuberculosis (H37Ra)

Unsensitized guinea pigs: From three hours to three days the inflammatory response was similar to that evoked by H37Rv. *By four days* the inflammation was subsiding, the monocyte clusters were small, and no extracellular multiplication of bacilli was evident. *At eight days* the tubercles were scanty and were smaller than those seen at this stage in the animals which had received H37Rv. *At ten days* the only bacilli that could be found were in the perivascular cuffs of cells. There were a few tubercles elsewhere which showed no development. No lymphocytes were seen (figure 11). *At thirty-two days* remnants of monocyte clusters were the only abnormalities which could be seen.

Guinea pigs previously sensitized with H37Rv: At four hours the omenta of the

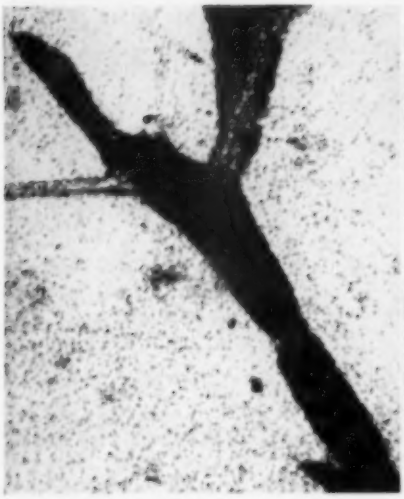

FIG. 11

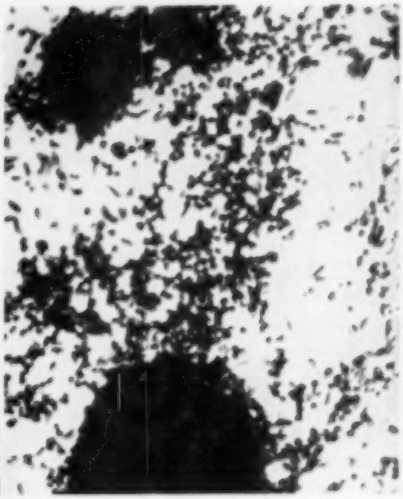

FIG. 12

FIG. 11. Guinea pig omentum ten days after inoculation with H37Ra. Perivascular hyperplasia is all that remains of reaction.

FIG. 12. "Sensitized" guinea pig omentum three hours after inoculation with H37Ra. Clumps of monocytes are larger than those in unsensitized omentum.

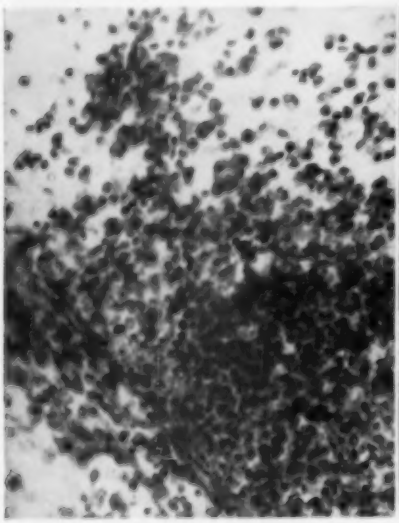

FIG. 13

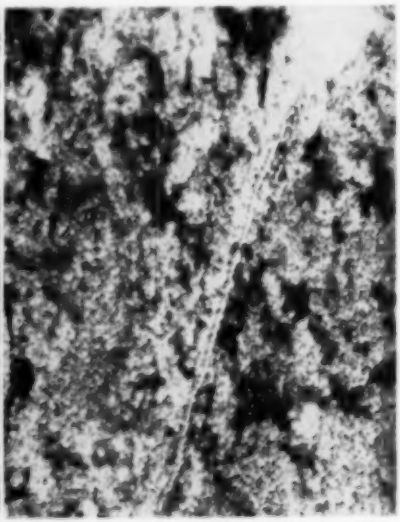

FIG. 14

FIG. 13. "Sensitized" guinea pig omentum three days after inoculation with H37Ra. Tubercle larger than those in unsensitized omentum at three days.

FIG. 14. "Sensitized" guinea pig omentum four days after inoculation with H37Ra.

sensitized animals challenged with the attenuated H37Ra strain showed clusters of monocytes which were larger and denser than those seen in the unsensitized pigs (figure 12). *At three to four days* there were dense tubercles infiltrated and surrounded by lymphocytes (figures 13, 14). *By nine days* the reaction was subsiding but was still the same pattern.

M. Tuberculosis BCG

Unsensitized guinea pigs: At three hours the reaction produced in unsensitized animals by BCG was indistinguishable from that produced by H37Rv. In figure 15 a typical cluster of agglutinated monocytes in a three-hour omental preparation is shown; some of the monocytes have phagocytosed bacilli. *At one day* the inflammatory reaction to BCG was likewise similar to that observed with H37Rv. *From the second to the eleventh day* perivascular proliferation was the most striking feature. There were a few tubercles observed at a distance from blood vessels, but most cellular activity was adjacent to the vessels. Bacilli were numerous in the perivascular tissue. *At sixteen days* tubercles adjacent to the blood vessels had formed dense cuffs or outgrowths (figure 16). A few lymphocytes were present, but most of the cells were monocytes. *At twenty-one days* the lymphocytes appeared to be more numerous and to be invading tubercles (figure 17). *By thirty-two days* perivascular cellular activity was all that remained. Bacilli were still demonstrable adjacent to the blood vessels.

Guinea pigs previously sensitized with H37Rv: At three hours in the H37Rv-sensitized animals challenged with BCG, there were more monocytes which formed larger clusters and enveloped more bacilli than were seen in the unsensitized omentum of animals similarly challenged. *At four days* there were a few dense tubercles surrounded and invaded by lymphocytes. *At seven days* the findings were essentially unchanged and no necrosis could be seen.

M. Smegmatis

Unsensitized guinea pigs: At three hours after the introduction of *M. smegmatis* there were fewer monocytes and more neutrophils than were found in three-hour omental specimens after infection with H37Rv, H37Ra, or BCG. A slight clumping of neutrophils was observed. *At two days* the tubercles and inflammatory reaction produced by *M. smegmatis* were greater than were observed in the two-day omenta infected with the other strains. *At four days* the inflammatory changes were still severe but *by ten days* the reaction was subsiding although many neutrophils persisted. *At thirteen days* a few scattered tubercles were still present along with occasional neutrophils but no lymphocytes. *At eighteen days* bacilli were still to be seen near blood vessels. Long perivascular sleeves of cells were the only abnormalities demonstrable (figure 18). The hyperplasia of the perivascular tissue could still be seen *at twenty-eight days* but the tissues were otherwise normal, and *by fifty-six days* no abnormalities at all were demonstrable.

Guinea pigs sensitized with H37Rv: Three hours after H37Rv-sensitized animals were challenged with *M. smegmatis*, the omental spreads showed clusters of monocytes and neutrophils around bacilli, which appeared to have been "rounded up." Very few free bacilli were noted (figure 19). *At four days* the cellular activity

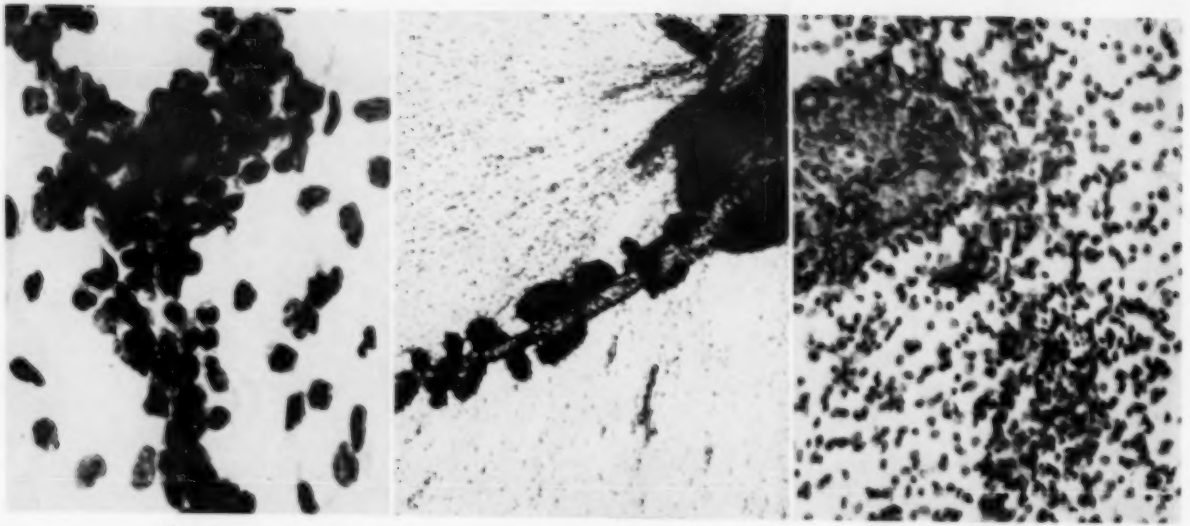

FIG. 15 FIG. 16 FIG. 17

FIG. 15. Guinea pig omentum three hours after inoculation with BCG. A clump of monocytes, some of which contain bacilli. This picture would do equally well for any of the strains three hours after inoculation.

FIG. 16. Guinea pig omentum sixteen days after inoculation with BCG. Perivascular tubercles; omentum otherwise relatively normal.

FIG. 17. Guinea pig omentum twenty-one days after inoculation with BCG. Lymphocytes and neutrophils around a small tubercle.

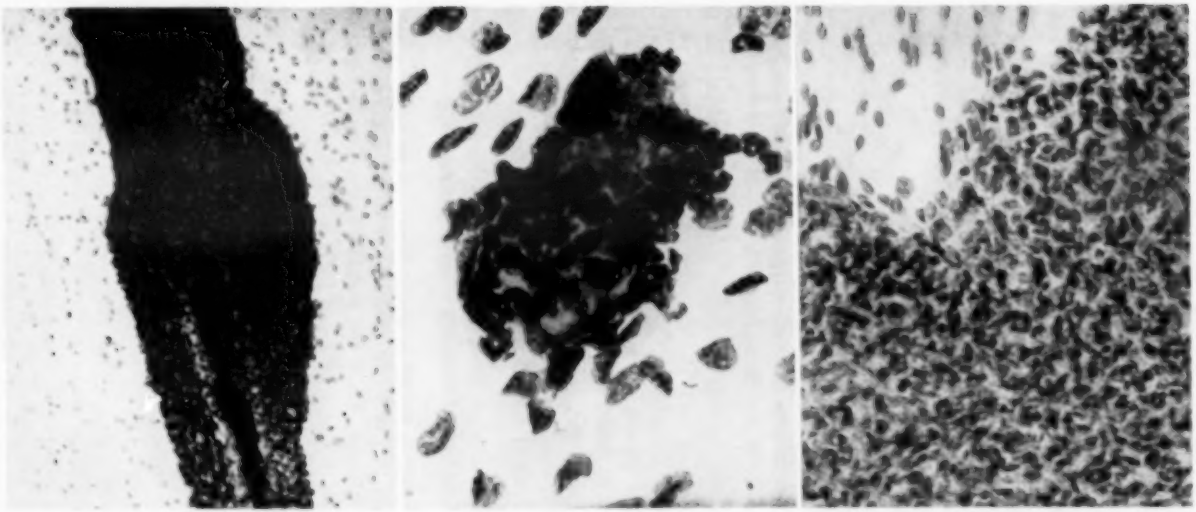

FIG. 18 FIG. 19 FIG. 20

FIG. 18. Guinea pig omentum eighteen days after inoculation with *M. smegmatis*. A perivascular "sleeve" of cells is all that remains.

FIG. 19. "Sensitized" guinea pig omentum three hours after inoculation with *M. smegmatis*, showing agglutination of monocytes, some of which have phagocytosed bacilli.

FIG. 20. Guinea pig omentum three days after inoculation with H37Ra. Guinea pig made leukopenic by nitrogen mustard. A typical tubercle without neutrophils or lymphocytes. Connective tissue cells appear to proliferate under these circumstances.

was concentrated at the omental margins and along blood vessels. The amount of cellular activity was more than that seen in the unsensitized pig inoculated with *M. smegmatis* but less than was seen in the sensitized pig four days after challenge with H37Ra. *At nine days* there was little change from the earlier findings. No lymphocytes were present and there was no necrosis.

M. Phlei and a Strain of Acid-Fast Bacilli Isolated from Tap-Water

Only a few guinea pigs were inoculated with *M. phlei*. Woodruff's observation that the formation of tubercles after inoculation with *M. phlei* occurs earlier than after inoculation with H37Rv was confirmed. The bacilli disappeared after three days.

In the animals inoculated with a strain of acid-fast bacilli isolated from tap-water, a few bacilli could be found up to three days in the omental preparations. The bacilli had all been ingested by neutrophils. No further development of the process was noted.

Omental Changes in Rabbits Following Intraperitoneal Inoculation of H37Rv and H37Ra

It is hoped to report the omental changes observed in rabbits in greater detail in a later paper dealing with the survival of tubercle bacilli of human origin in rabbits. Suffice it to state at this time that a rapid proliferation of host cells occurred in the omentum of sensitized rabbits. By four days this proliferation had become so dense that individual cells were indistinguishable. In the rabbit as in the guinea pig, the early appearance of lymphocytes served to distinguish the omental changes seen after reinfection with either H37Rv or H37Ra of an H37Rv-sensitized animal from the changes present in a primary infection.

Development of Tuberculin Hypersensitivity

In the present experiments in guinea pigs, all sensitized animals had been infected with H37Rv within two or three weeks prior to being given their intraperitoneal challenge inoculation. All of the animals showed a strongly positive reaction to the intradermal injection of 2 γ of PPD in 0.1 mg. buffer at the time of challenge.

Most guinea pigs primarily infected by the intraperitoneal inoculation of H37Rv gave positive reactions towards the end of the first week. Additional animals primarily inoculated with saprophytic strains or with H37Ra did not give positive reactions to 20 γ of PPD. Sensitization with BCG produced milder tuberculin reactions than sensitization with H37Rv, and the cutaneous hypersensitivity took longer to develop.

One batch of 12 pigs was younger than the average and the pigs weighed between 180 and 200 gm.; for three weeks they were deprived of their normal pellet diet and were fed only on green stuff. Consequently they did not gain in weight. It was found that these animals remained tuberculin-negative for four weeks after their intraperitoneal inoculations. Those killed at the end of the thirteenth,

fourteenth, nineteenth, and twenty-second days showed tubercles on the omentum but no lymphocytes and no necrosis. It was only after they became tuberculin-positive on the thirtieth day that lymphocytes were found in and around the tubercles and only in these was necrosis apparent.

The Effect of Nitrogen Mustard on the Tuberculous Process

Because there appeared to be a temporal relationship between hypersensitivity, lymphocytosis, and necrosis of tubercles, it was decided to investigate the effect of suppressing lymphocytes by administration of the nitrogen mustard CB 1348, which was known to produce lymphopenia in rats, though its action in guinea pigs was uncertain.

Twelve guinea pigs were given nitrogen mustard and were subsequently inoculated intraperitoneally. Half of them had been sensitized previously with H37Rv. Unfortunately, the mustard produced a neutropenia rather than lymphopenia. In the animals which survived as long as a fortnight, there was extensive fatty degeneration of the liver so that the experiment was abandoned. Monocytes clumped and formed tubercles of a sort (figure 20), but these remained sheets of cells rather than tubercles. Connective tissue cells appeared to proliferate more in the sensitized than in the unsensitized omenta. Tubercle bacilli of the H37Ra strain were also inoculated into guinea pigs made leukopenic by the nitrogen mustard. There was no evidence of any longer survival of the bacilli in the relative absence of leukocytes.

DISCUSSION

Monocytes and, to a lesser degree, neutrophils, which have ingested bacilli, agglutinate. On these raised and sticky clumps of cells more bacilli and cells adhere. These clumps of monocytes appear to be the beginning of tubercles (4), although tubercles also form among the fixed macrophages of the perivascular areolar tissue.

The first point to be emphasized is that, with mycobacteria, this agglutination of phagocytes is greatly enhanced in previously sensitized animals and that sensitization with H37Rv leads to larger clumps of agglutinated phagocytes even when other strains are used for challenge. The effect of vaccination with nonpathogenic strains on this phagocytic agglutination phenomenon has not been studied.

Following the intraperitoneal introduction of mycobacteria, neutrophils pour out from the capillaries at the time of the inflammatory reaction which begins between the first and second hours. The number of neutrophils in the peritoneal exudate increases during the period when bacilli can be found multiplying extracellularly on the omental surface, and they disappear as the bacilli disappear. The disappearance of free corded bacilli coincides with the increasing neutrophilia. Many of the neutrophils which have phagocytosed bacilli are subsequently ingested by monocytes and many of the bacilli which arrive at the tubercles appear to have been conveyed in neutrophils. Ingestion of bacilli in cords by monocytes has not been observed. The question arises whether a lipase

from neutrophils might be responsible for separating the individual cells in the "cords" and hence for the subsequent absence of the corded pattern.

After the introduction of attenuated or avirulent strains of mycobacteria there are sheets of cells on the omentum which never become crowded after the initial inflammatory reaction has subsided. They gradually disappear after the first week, but the bacilli with them disappear in two or three days. In contrast, *M. smegmatis* may be found in proximity to the capillaries after two weeks. These observations suggest that the survival of bacilli *in vivo* as *in vitro* may be related to their need for oxygen; that in the most oxygen-dependent strain, *M. phlei*, survival *in vivo* is of very short duration—perhaps no longer than the initial phagocytosis and envelopment in a tubercle—but that *M. smegmatis* survives longer close to capillaries. The perivascular hyperplasia which is so striking a feature of the three-week omentum infected with *M. smegmatis* persists when all else has subsided. It is possible that the longer activity of perivascular cells is evidence of longer survival of bacilli in these areas.

The BCG strain displays the growth behavior *in vitro* of a virulent rather than an attenuated or avirulent strain of organisms under low oxygen tension (5). In the present studies, the cells of the BCG strain persisted longer in tubercles away from capillaries than did the avirulent strains. After sensitization, however, and the lymphocytic invasion of the tubercles in the third week, further activity was restricted to the perivascular tubercles (figure 16). In the previously sensitized animal, the BCG cells disappeared from the omental lesions during the first week of infection. The BCG organisms appeared to survive phagocytosis and the mild congestion of the "open" tubercle better than the avirulent strains, but did not survive in the crowded and necrotic tubercles as was the case with the fully virulent strains. It is possible that BCG would be more effective as a vaccine if it were inoculated into highly vascular rather than into relatively avascular tissue.

In the normal guinea pig, bacilli increase in the tubercles until the end of the third week, after which there is a decline as necrosis and caseation ensue. This change in the numbers of bacilli appears to be related to the cell density of the tubercles which occurs after the lymphocytic invasion. Where there is less crowding, bacilli still appear to be multiplying. Loebel, Shorr, and Richardson (6) calculated that the center of a tubercle with a radius of 0.075 mm. to 0.35 mm. is anaerobic and that it takes three or more days of complete anaerobiosis to destroy the capacity of virulent bacilli to multiply. The monocytes survive because they have an alternative source of energy—they can convert glucose to lactic acid.

In the reinfected animal the inflammatory reaction and cellular proliferation are so much greater that there is as much congestion in four days as develops in the primarily infected animal in three weeks. There is no "free growth period" in the previously sensitized guinea pig (1) and lymphocytosis is apparent from the first day (3). There seems to be no need to postulate immunity mechanisms other than this far greater and more rapid cellular response to secondary infection to explain the absence of free bacillary growth.

A study of the cytology of the tubercles shows that lymphocytes begin to appear approximately ten days after inoculation with H37Rv and a little later with BCG. Lymphocytes did not appear within the period of observation in the present experiments with H37Ra, *M. smegmatis*, *M. phlei*, or the tap-water strain. As Gardner (2) points out, the increase of lymphocytes coincides with the development of hypersensitivity.

Jensen, Bindslev, and Holm (7), in their study of the development of tuberculous infection of the lungs in allergic and nonallergic guinea pigs, found a peripheral accumulation of round cells on the fifteenth day in the lesions of nonallergic pigs, which by the twentieth day had become "streaks of round cells extending from the margin towards the center." They did not relate necrosis of tubercles to the presence of these cells.

There is some experimental evidence for the theory that antibody to tuberculoprotein is carried on lymphocytes. Favour (8) claims that "Cell destruction in man and guinea pigs by tuberculin involves lymphocytes, which make antibody, and other cells which are capable of absorbing tuberculin, viz., neutrophils." Wesslen (9) found that between 20 and 30 per cent of lymphocytes from tuberculous guinea pigs lysed when incubated with tuberculin.

The observations made in the present study lead to the following hypothesis:

> In primary infection the avirulent strains do not survive long enough to sensitize the host under the conditions of the experiment. BCG and H37Rv do multiply sufficiently to provide enough antigen for sensitization. Antibody is carried by lymphocytes which increase in number as more bacilli are destroyed and more antigen is released. The invasion of the lymphocytes itself increases the rate of destruction of bacilli by increasing the density of the tubercles. The combination of the antigen, released from lysing bacilli, with the antibody on sensitized lymphocytes leads to the necrosis of tubercles. The caseation of the thirty-second day omentum (figures 6, 7) appears to be an extension of this process. The lymphocytes which surround caseous foci appear to be survivors at the periphery of areas where an abrupt necrosis has occurred and in which similar lymphocytes have been involved.
>
> In the reinfected guinea pigs, H37Rv and BCG produce lymphocytosis within twenty-four hours and necrosis occurs within a week.

One way of adding support to the hypothesis that necrosis is brought about in this way would be to demonstrate the absence of necrosis in the absence of lymphocytes, and it is unfortunate that the nitrogen mustard experiments proved unfruitful. The converse, to show an acceleration of necrosis when sensitized lymphocytes are introduced before they would normally appear, would be less satisfying, but further experiments are being planned along these lines.

There appear to be four stages in the local defence tactics: phagocytosis, the aggregation of phagocytes to form tubercles, the invasion by lymphocytes, and the necrosis of tubercles. The first two stages are sufficient to terminate infection by "avirulent" strains. The third terminates infection by the "intermediate" strain BCG, while the virulent strains may survive, though not multiply, in the caseous necrotic tissue. Dubos (10) has recently stated that "natural and ac-

quired resistance depends more often upon the ability of the inflammatory response to create in and around the lesion a micro-environment inimical to the infectious agent." The present study is concerned with the tissue changes which produce the micro-environment upon which natural and acquired resistance to the mycobacteria depends.

SUMMARY

The changes in the omenta of guinea pigs and rabbits following intraperitoneal inoculation of *M. tuberculosis*, H37Rv, H37Ra, BCG, *M. smegmatis*, *M. phlei*, and an unspecified mycobacterial strain obtained from tap-water have been described.

Attention has been drawn to the immediate agglutination of monocytes and neutrophils which have phagocytosed bacilli and to the enhancement of this phenomenon in reinfected animals.

It is suggested that the survival of bacilli and the persistence of lesions may correspond, among other factors, to their ability to multiply *in vitro* in an environment deficient of oxygen.

An invasion by lymphocytes is apparent at the time hypersensitivity occurs in primary infections and in about two days after secondary infection. It is suggested that the cell density of tubercles may restrict bacillary multiplication and that subsequent necrosis is brought about by a combination of antigen released from bacilli with antibody carried by lymphocytes.

SUMARIO

La Patogenia de la Tuberculosis Según la Revelan las Propagaciones Epiploicas

Descríbense las alteraciones observadas en los epiplones de cobayos y conejos consecutivamente a la inoculación intraperitoneal de *M. tuberculosis*, H37Rv, H37Ra, BCG, *M. smegmatis*, *M. phlei* y de una cepa micobacteriana no especificada, obtenida del agua del grifo.

Llámase la atención sobre la aglutinación inmediata de monocitos y neutrófilos que contienen bacilos fagocitados y sobre el realce de este fenómeno en los animales reinfectados.

Sugiérese que la sobrevivencia de los bacilos y la persistencia de las lesiones tal vez corresponda, entre otros factores, a su capacidad para multiplicarse *in vitro* en un ambiente carente de oxígeno.

La invasión por linfocitos es manifiesta para la fecha en que sobreviene hipersensibilidad en las infecciones primarias y aproximadamente unos dos días después de la infección secundaria. Sugiérese que la densidad celular de los tubérculos quizás limite la multiplicación bacilar y que el esfacelo subsiguiente es producido por una combinación del antígeno desprendido de los bacilos con el anticuerpo conducido por los linfocitos.

RESUME

La pathogénie de la tuberculose observée dans l'étude des diffusions épiploïques

Description des modifications de l'épiploon chez les cobayes et les lapins à la suite de l'inoculation intrapéritonéale de *M. tuberculosis*, H37Rv, H37Ra, BCG, *M. smegmatis*, *M. phlei* ainsi que celle d'une souche mycobactérienne non spécifiée prélevée dans l'eau du robinet.

L'attention est attirée sur l'agglutination immédiate des monocytes et des neutrophiles ayant phagocyté les bacilles et l'exagération de ces phénomènes chez les animaux réinfectés.

L'article suggère que la survie des bacilles et la persistance des lésions peuvent, entre autres facteurs, correspondre à leur faculté de se reproduire, *in vitro*, dans un milieu pauvre en oxygène.

L'invasion par les lymphocytes devient apparente au moment où l'allergie se produit dans les infections primaires et environ deux jours après dans l'infection secondaire. Il est suggéré que la densité cellulaire des tubercules peut restreindre la reproduction bacillaire et que la nécrose qui survient ensuite est provoquée par l'action combinée de l'antigène libéré par les bacilles et de l'anticorps transporté par les lymphocytes.

REFERENCES

(1) Woodruff, C. E.: A free growth period of tubercle bacilli in the guinea pig omentum as related to the hypersensitivity state, Am. J. Path., 1934, *10*, 739.

(2) Gardner, L. U.: Studies on the tissue reactions to primary infection and reinfection with the tubercle bacillus: I. A histological examination of the omentum and other subperitoneal tissues, Am. Rev. Tubere., 1929, *20*, 201.

(3) Gardner, L. U.: The cellular reactions to primary infection and reinfection with the tubercle bacillus: II. The cells of peritoneal exudate produced by the local injection of tubercle bacilli into normal and sensitized guinea pigs. Am. Rev. Tubere., 1930, *22*, 379.

(4) Woodruff, C. E., Kelly, R. G., and Leaming, M. A.: Initial tissue response to tubercle bacilli, Am. Rev. Tubere., 1942, *46*, 319.

(5) Guy, L. R., Raffel, S., and Clifton, C. E.: Virulence of the tubercle bacillus. II. Effect of oxygen tension upon growth of virulent and avirulent bacilli, J. Infect. Dis., 1954, *94*, 99.

(6) Loebel, R. O., Shorr, E., and Richardson, H. B.: The influence of adverse conditions upon the respiratory metabolism and growth of human tubercle bacilli. J. Bact., 1933, *26*, 167.

(7) Jensen, K. A., Bindslev, G., and Holm, J.: Experimental studies on the development of tuberculous infection in allergic and non-allergic animals, Acta. tubere. Scandinav., 1935, *9*, 27.

(8) Favour, C. B.: *In vitro* effect of tuberculin on cells, Advances in Tuberculosis Research, S. Karger, Basel, 1951, p. 219.

(9) Wesslen, T.: *In vitro* studies on tuberculin hypersensitivity of lymph cells, Acta dermat. Venereol., 1952, *32*, 195.

(10) Dubos, R. J.: The micro-environment of inflammation, Lancet, 1955, *2*, 1.

CHRONIC TUBERCULOUS INFECTION IN EXPERIMENTAL ANIMALS[1]

CHARLES H. LACK

(Received for publication September 6, 1955)

INTRODUCTION

In an investigation of the pleomorphism of *M. tuberculosis*, part of which has already been published (1), bacilli were inoculated intradermally into guinea pigs, rabbits, and mice, and lesions were excised for study over long periods. Such full descriptions have already been published on the sequence of changes in the skin of these animals following intracutaneous infection that it is not proposed to give full histologic reports of this experimental material. Instead, it is proposed to draw attention to the chronicity and other aspects of this type of infection not emphasized by previous writers. Attention is also drawn to the long survival of bacilli of human origin in rabbits' lungs and kidneys and to the activating effect of cortisone on chronic lesions in one animal.

MATERIALS AND METHODS

Throughout these experiments, the H37Rv human strain of *M. tuberculosis* was employed. It was grown in liquid Dubos Tween®-albumin medium for two weeks, spun down, weighed wet, and resuspended in fresh medium immediately before use. The bacilli were suspended in a concentration of 1 mg. wet weight per ml., and all inoculations consisted of 0.1 ml. of suspension injected intradermally into the shaved skin of the back.

Ten adult guinea pigs were each inoculated in six sites, 32 mice were each inoculated in only one site, and 25 adult rabbits, weighing between 3 and 4 kg., were each inoculated in ten sites.

The lesions were excised at intervals, the animals being used in rotation to provide these biopsies. The wounds were closed with Michel clips. In addition to smears, culture, and fixation for histologic study, portions of biopsy material were occasionally pouched into thighs of guinea pigs. Paraffin sections were stained with hematoxylin-eosin, Ziehl-Neelsen, and auramine-rhodamine.

RESULTS

Guinea Pigs

Intradermal lesions: In a representative experiment, collections of neutrophils and macrophages appeared in the inoculation site within the first forty-eight hours and an abscess had formed by the fourth day. There was little evidence of localization of the infection during the first week (figure 1) and fluorescent microscopy showed a wide dispersal of the bacilli with an apparent increase in the inflammatory area up to the tenth day.

By the fourteenth day the abscess, now 1 cm. in diameter, had begun to slough (figure 2). Around it, neutrophils, monocytes, macrophages, and lymphocytes extended into the underlying muscle. During the third week hemorrhages

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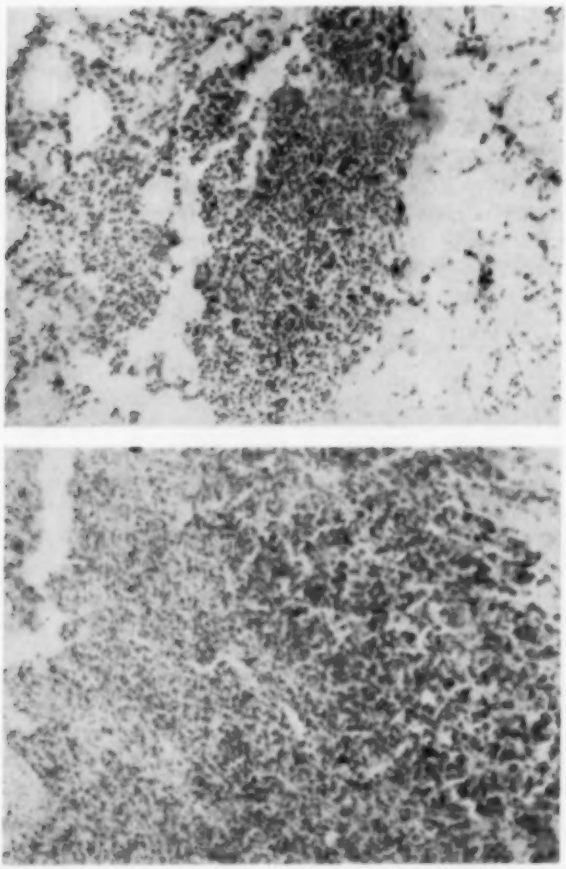


FIG. 1. (Top) Guinea pig skin: Sixth day. Abscess forming at inoculation site. (H. & E.)

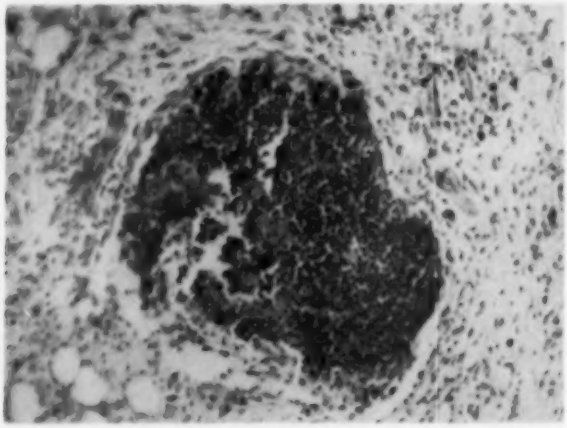
FIG. 2. (Bottom) Guinea pig skin: Fourteenth day. Necrotic abscess starting to slough. (H. & E.)

were conspicuous and necrosis extended down to the muscle. During the fourth week caseous foci were found in the dermis well away from the inoculation site. Relatively few bacilli could be found in the thirty-day sections and those were mainly in the connective tissue and muscle planes. The bacilli throughout this period were of normal morphology and many were in an inverted form suggesting division. No multiplication in cords was observed.

Lesions elsewhere: No study was made of the spread of bacilli to other organs, but it was noted that the spleen enlarged to twice the normal size during the third week, by the end of which time it showed microscopic hemorrhages and caseation.

Mice

Intradermal lesions: In a representative experiment with the intradermal infection, an abscess formed of neutrophils was well developed at the inoculation site by the second day and by the fourth day was well circumscribed (figure 3). By the sixth day the abscess had become necrotic and there was considerable fibrinous exudate. By contrast with the lesions in the guinea pig at this stage, the bacilli were mostly contained within the abscess and there was surprisingly little inflammatory reaction around it (figure 4). On the twelfth day macrophages and monocytes and a few multinucleated cells were found in the adjacent dermis. The inflammatory reaction in the underlying connective



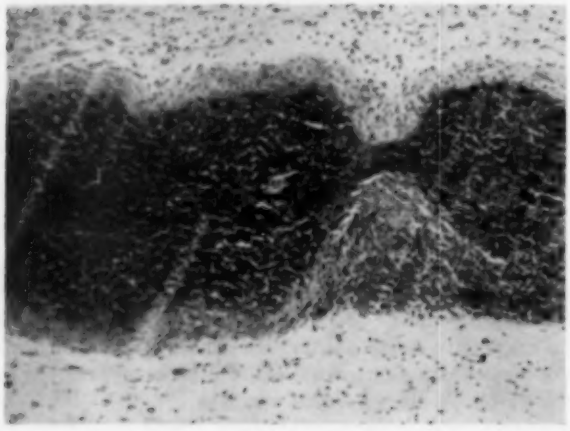


FIG. 3. (Top) Mouse skin: Fourth day. Hyaline capsule around abscess; little peripheral reaction. (H. & E.)

FIG. 4. (Bottom) Mouse skin: Sixth day. The lesion is still well localized; the bacilli are confined within the abscess. (H. & E.)

tissue slowly increased and foam cells became common by the sixtieth day, but were not as numerous as the macrophages. The abscess was still present, although smaller, on the eightieth day and there had been some sloughing of the epidermis. Examination of the last biopsy specimen on day 186 showed liquefaction of the abscess contents, that there were no bacilli within it, and that there were short fat bacilli in the neighboring connective tissue.

A portion of the specimen obtained by biopsy on day 186 was cultured in Dubos medium to which penicillin had been added in a concentration of 100 units per ml. In four months a growth of short acid-fast bacilli was obtained and subcultured onto Löwenstein's medium, which grew as typical *M. tuberculosis*. Pleomorphism of the bacilli was a notable feature of these mouse lesions, and Ziehl-Neelsen- and Giemsa-stained sections showed far more granular forms and coccal forms than were apparent with fluorescent microscopy. The Giemsa-stained bacilli were eosinophilic. Dispersal into adjacent tissue was very slow by comparison with the spread in the guinea pig. Other organs were not examined in this series of mice.

The localization of the infection occurred earlier and was apparently more effective in mice than in guinea pigs or rabbits. This was not due to earlier fibrosis but to a hyaline barrier which was conspicuous from the fourth day (figure 3).

Tubercles were not seen, lymphocytes did not infiltrate the lesion, and caseation was not observed at any stage in the cutaneous lesion. The absence of a reactive zone may have contributed to the survival of the bacilli for as long as 186 days.

Rabbits

Intradermal lesions: The histologic changes in the rabbit skin lesions were similar to those described by Feldman and Mann (2) for the first forty days; in the present study, biopsies were made until day 195.

The dermal lesion may best be described as a purulent reaction at the site of infection followed by a granulomatous process spreading outwards from the margin of the abscess. In a representative experiment, neutrophils, the first phagocytes on the scene, formed the abscess (figure 5), and the surrounding cells were mainly histiocytes, macrophages, and (later) epithelioid cells and giant cells. Lymphocytes infiltrated the lesion between the eighth and seventeenth days and were seen in and around the caseating areas (figure 6). The abscess usually sloughed about this time (figure 7). The ulcer gradually filled in by fibrosis while the surrounding tissue between the third and fifth months showed relatively little change. By day 195, the lesion was mostly scar tissue.

The number of bacilli in the skin lesions appeared to be fairly constant until approximately the seventeenth day, after which time there was a marked drop.

This fall in numbers coincided with the onset of focal necrosis away from the abscess and with sloughing of the abscess itself. Between day 30 and day 195, the bacilli observed by fluorescent microscopy were shorter than normal and were very scanty. Portions of the skin biopsies taken on day 133 and on day 150 were inserted into muscle pouches in guinea pigs and both produced tuber-

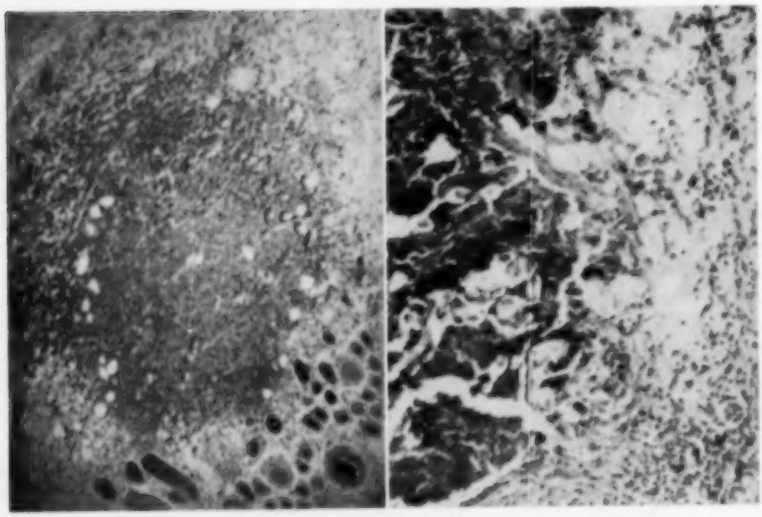


FIG. 5. (Left) Rabbit skin: First day. Abscess forming at inoculation site. (H. & E.)
FIG. 6. (Right) Rabbit skin: Seventeenth day. The base of a sloughing abscess. (H. & E.)

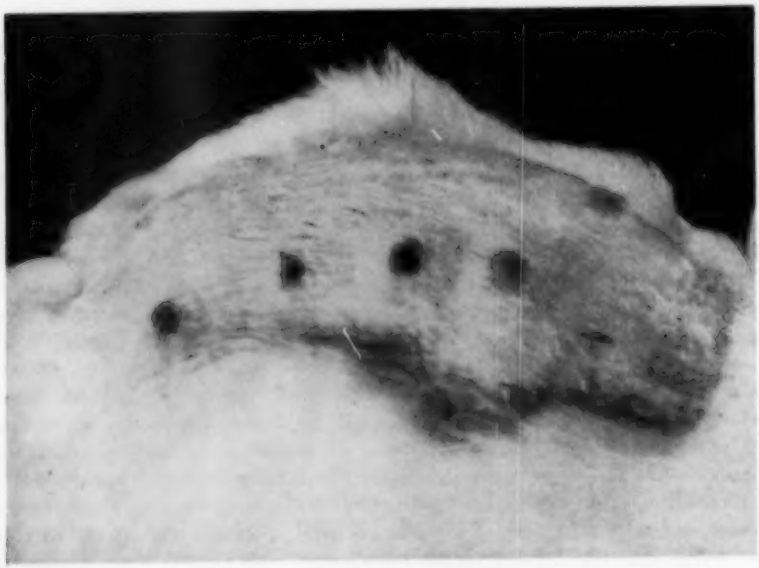
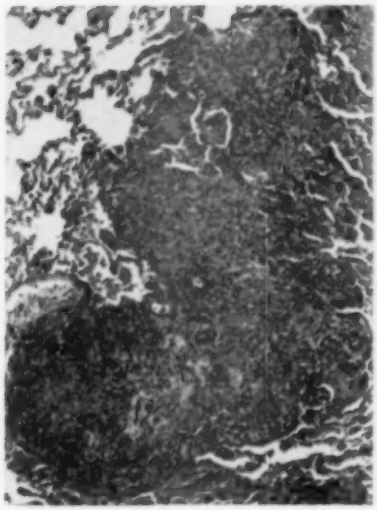


FIG. 7. Rabbit on seventeenth day, showing intracutaneous lesions beginning to ulcerate.



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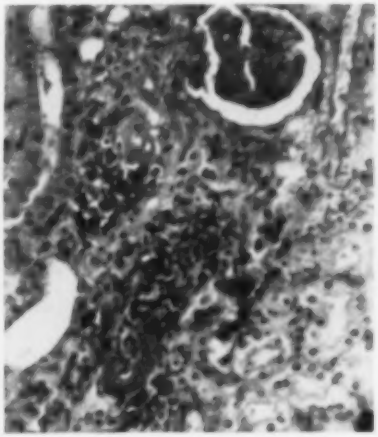

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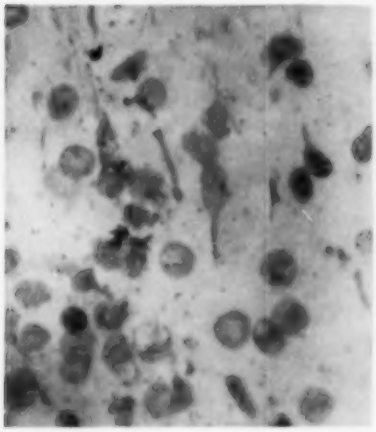

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FIG. 8. Rabbit lung: Day 158. Typical "hard" tuberculous focus. (× 100)

FIG. 9. Rabbit kidney: Day 158. A "nonspecific" lesion containing acid-fast bacilli. (× 100)

FIG. 10. Rabbit kidney: Day 158. Smear of kidney showing clusters of short acid-fast bacilli. (Ziehl-Neelsen, × 450)

FIG. 11. Rabbit lung: Day 195. Caseous nodule on lung surface containing demonstrable acid-fast bacilli. (Ziehl-Neelsen, × 100)

TABLE 1

Days After Inoculation	Death	Post-Mortem Findings
1	Killed by air embolism	Lung, kidney, and spleen homogenized and digested with trypsin; lung and spleen contaminated and discarded. Kidney yielded culture of acid-fast bacilli.
17	Chloroform	Heart blood injected into guinea pig did not infect. Lungs, kidneys, liver, spleen showed no macroscopic or microscopic lesions.
44	Chloroform	Lungs contained numerous tubercles.
47	Chloroform	Viscera normal.
63	Chloroform	Viscera normal.
72	Chloroform	Tuberculous foci with demonstrable acid-fast bacilli in lungs, liver, spleen, and kidney. Portion of skin lesion inserted into muscle of guinea pig produced extensive tuberculosis.
77	Chloroform	Lesions in lungs and kidney but not found in spleen or liver.
133	Chloroform	Tuberculous foci with demonstrable acid-fast bacilli in lungs and liver. Spleen and kidneys not examined. Portion of skin lesion inserted into muscle of guinea pig produced extensive tuberculosis.
158	Died	Caseous foci 2 mm. in diameter in lung (figure 8); guinea pig test positive. Lesion in kidney (figure 9) and smears of fresh kidney tissue (figure 10) showed numerous short acid-fast bacilli. Guinea pig test negative.
195	Died fighting	Caseous foci 1 to 2 mm. in diameter mostly on lung surface (figure 11). Kidneys contain numerous small collections of epithelioid cells. No acid-fast bacilli found in kidney and no lesions found in spleen or suprarenal.
304	Died after fighting	Sites of inoculation in this animal had been excised within the first six weeks. No histologic evidence of tuberculosis found in lungs, kidneys, spleen, liver, or suprarenal. Portion of lung and kidney inserted into muscle of guinea pig: negative. One kidney had staphylococcal abscess.
366	Died	Lungs showed chronic tuberculous foci characterized by giant cells and epithelioid cells, without much fibrosis. No tubercles were found in kidney, but occasional clusters of monocytes.
409	Chloroform	Lungs contain nodules 2 to 3 mm. in diameter, containing tubercle bacilli. Other viscera normal.

culosis. No guinea pig tests were made with biopsy specimens obtained at a later date.

Lesions elsewhere: Post-mortem examinations were made on most of the rabbits in this series and the findings are summarized in table 1.

When it was found by these guinea pig inoculations that bacilli remained viable in the skin, lungs, and kidneys for this long period of time, it was decided to observe the effect of cortisone on these chronic lesions.

Action of Cortisone on Chronic Infection in a Rabbit

Two litter-mate rabbits were inoculated intradermally with tubercle bacilli (1 mg. of H37Rv) on the same day and both gave positive reactions, 3 cm. in diameter, to 1:1,000 PPD ninety-four days later. One hundred and twenty-six

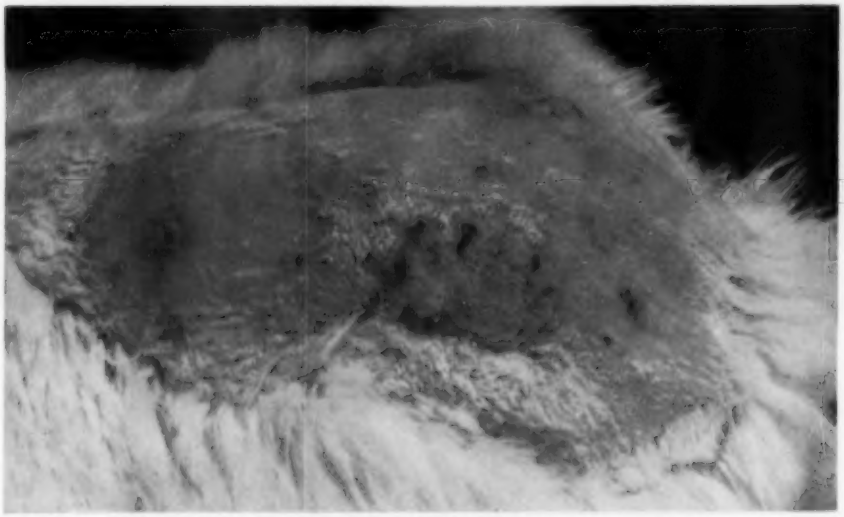


FIG. 12. Control rabbit 126 days after intracutaneous inoculation of H37Rv; two lesions and the reaction to PPD can be seen.

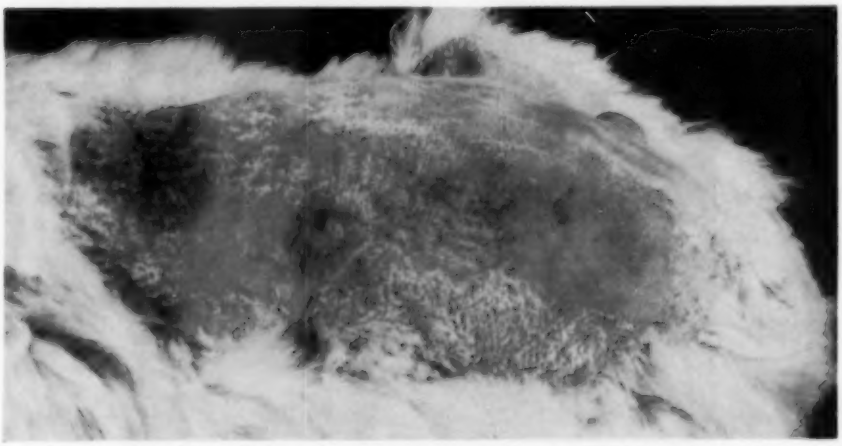


FIG. 13. Test rabbit 126 days after intracutaneous inoculation of H37Rv; two lesions, one in profile, can be seen. This rabbit was started on cortisone on the day this photograph was taken.

days after the initial inoculation, one rabbit was given cortisone acetate, 25 mg. twice daily for fifteen days. The rabbits were isolated in separate cages throughout the experiment and both were killed on day 151 after infection and eleven days after the stoppage of cortisone in the test rabbit (figures 12, 13, and 14).

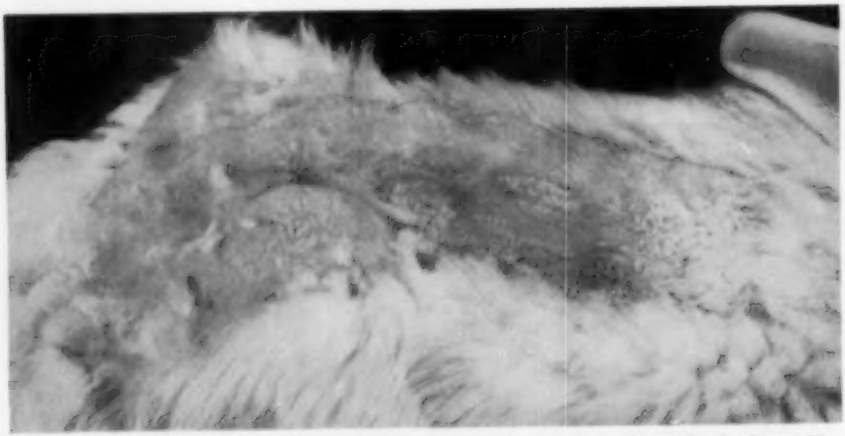
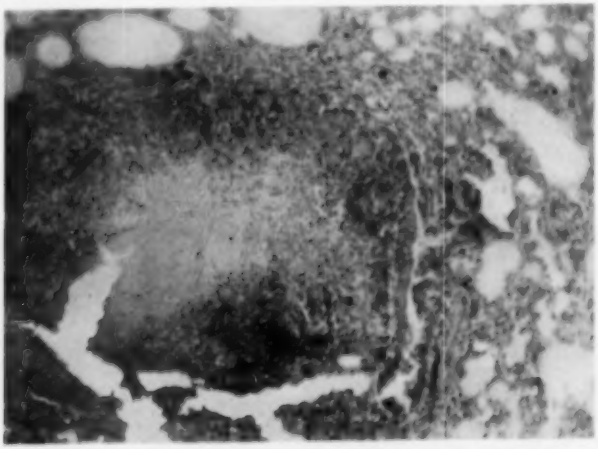


FIG. 14. Test rabbit after course of cortisone; the lesion shown in profile in figure 13 is in the foreground and is now a large fluctuant abscess.



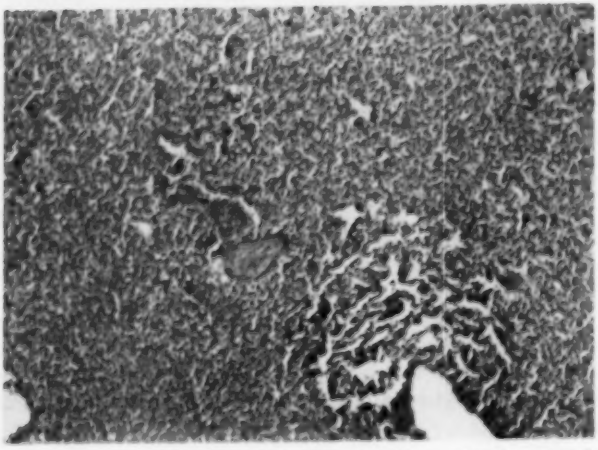


FIG. 15. (Top) Control rabbit lung on day 151 after infection a hard caseous focus. (× 100)

FIG. 16. (Bottom) Test rabbit lung on day 151 after infection; softening and dense infiltration of caseous focus in rabbit which had been given cortisone from day 126 to day 141. (× 100)

Post-mortem examination of control rabbit: On post-mortem examination, there were two firm papules at the sites of intradermal inoculation and "hard" tuberculous lesions in the lungs (figure 15). No lesions were found elsewhere.

Post-mortem examination of the cortisone-treated rabbit: On post-mortem examination, the cortisone-treated rabbit was emaciated. The skin lesions were soft and fluctuant. The lungs contained small acute abscesses which had the appearance of infiltrations of hard tuberculous lesions (figure 16). Numerous acid-fast bacilli were present. Both kidneys showed large polar abscesses which were full of fluid pus. There was no histologic evidence of tuberculosis, and no acid-fast bacilli were found in the liver, spleen, or suprarenal glands.

It was not possible to increase the number of rabbits for this test because only 2 of the batch with chronic infections could be exactly paired and had skin lesions of such long duration.

DISCUSSION

When the intracutaneous reaction in the three species of animal is compared, it is seen that abscess formation and its discharge onto the surface was common to all, although sloughing of the epidermis was much slower in the mouse than in the other species. Pagel (3) has commented on the localization of the bacilli, the absence of caseation and of tubercles, and the conversion of monocytes into foam cells in the mouse but makes no reference to lymphocytes. These cells always appear in the lesion in guinea pigs and rabbits about the same time hypersensitivity becomes apparent although they are found around—rather than in—the caseous foci. Lymphocytes are not a prominent feature in the lesions in the mouse. It seems clear that abscess formation at the site of inoculation and the granulomatous changes which occur peripherally are separate processes, varying in different species.

Contrasting the skin lesions with those observed in the omentum of rabbits and guinea pigs (4), the following differences are noted:

> In the skin the local response is more acute at the onset and the necrosis during the second and third weeks is accompanied by more vascular damage and hemorrhage. While neutrophils play a major role in both sites, monocytes also appear in large numbers within a few hours in the omentum but do not appear in the skin until much later. Lymphocytes are more noticeable in omental lesions at the time of sensitization, but this may be because they are easier to see in the looser tissue.

There is little information on which to base a comparison of the spread to, and survival in, other organs after intracutaneous infection.

Griffith and Griffith (5), Soltys and Jennings (6), and Desbordes (7) have reported the presence of bacilli in the lungs, liver, spleen, and kidneys within a few hours of intracutaneous inoculation in guinea pigs; no reference has been found to similar investigations in rabbits or mice. In the present investigation, tubercle bacilli were grown from the homogenized kidney of a rabbit which had been inoculated intracutaneously twenty-four hours previously; no attempt was made to recover bacilli from the urine.

The survival of tubercle bacilli of human origin for more than a year in chronic lesions in the rabbit lung was surprising. Some of these lesions showed no caseation but were nodules of epithelioid cells and giant cells with very little perifocal reaction. The chronicity and stability of the infection with human bacilli in rabbits offers one advantage over the use of mice as advocated by Hart and Rees (8). The host response to tuberculous infection and to hormonal disturbance in the rabbit approximates more closely that in man.

Many reports have been made on the enhancing effect of cortisone on experimental tuberculosis in animals (*see* list in reference (9)), but in most of these studies cortisone has been given at the onset of infection. Hart and Rees (8) found that cortisone given to mice with established chronic pulmonary infections caused enlargement and necrosis of what had been proliferative lesions, with an enormous increase in bacilli. The only justification for describing here the effect of cortisone on one rabbit is to draw attention to the use of intradermal infection with tubercle bacilli of human origin in the study of factors affecting chronic tuberculous infection.

SUMMARY

Differences in host response after intracutaneous inoculation of *M. tuberculosis* (H37Rv) into guinea pigs, mice, and rabbits are described. The tubercle bacilli may survive for more than a year in chronic lesions in the lungs of rabbits. The use of this experimental method for studying factors affecting the stability of chronic infections is suggested and observations on the adverse effect of cortisone are presented as an example.

SUMARIO

Infección Tuberculosa Crónica en los Animales de Experimentación

Se describen aquí las diferencias observadas en la reacción del huésped a la inoculación intracutánea de *M. tuberculosis* (H37Rv) en cobayos, ratones y conejos. Los bacilos tuberculosos pueden sobrevivir más de un año en las lesiones crónicas de los pulmones de conejo. Sugiérese el empleo de este método experimental para estudiar factores que afecten la estabilidad de las infecciones crónicas y se ofrecen, como ejemplo, observaciones acerca del efecto adverso de la cortisona.

RESUME

Infection tuberculeuse chronique chez l'animal en expérience

Description des différences observées dans la réaction de l'hôte après inoculation intradermique de *M. tuberculosis* (H37Rv) aux cobayes, souris et lapins. Le bacille tuberculeux peut survivre pendant plus d'une année dans les lésions chroniques du poumon des lapins. L'emploi de cette méthode expérimentale pour étudier les facteurs affectant la stabilité des infections chroniques est suggéré, et des observations sur l'effet adverse exercé par la cortisone sont présentées à titre d'exemple.

Acknowledgment

The writer wishes to acknowledge the technical assistance of Mr. F. A. Tanner in this work.

REFERENCES

(1) Lack, C. H., and Tanner, F. A.: The significance of pleomorphism in *M. tuberculosis* var. *hominis*, J. Gen. Microbiol., 1953, *8*, 18.

(2) Feldman, W. H., and Mann, F. C.: Intracutaneous tuberculosis in rabbits, Am. Rev. Tuberc., 1939, *40*, 336.

(3) Pagel, W.: Experimental tuberculosis, Am. Rev. Tuberc., 1940, *42*, 58.

(4) Lack, C. H.: The pathogenesis of tuberculosis as shown in studies of omental spreads, Am. Rev. Tuberc., 1956, *73*, 362.

(5) Griffith, S., and Griffith, F.: Report of the Royal Commission on Tuberculosis, 1907, *3*, 237.

(6) Soltys, M. A., and Jennings, A. R.: The dissemination of tubercle bacilli in experimental tuberculosis in the guinea pig, Am. Rev. Tuberc., 1950, *61*, 399.

(7) Desbordes, J.: Application pratique des bactéries marquées: Etude de la vitesse de dispersion du *Mycobacterium tuberculosis* marqué au ^{32}P chez le cobaye. Sort précoce des bacilles en la présence ou en l'absence d'hyaluronidase, Compt. rend. Soc. de biol., 1953, *147*, 1212.

(8) Hart, P. D'A., and Rees, R. J. W.: Enhancing effect of cortisone on tuberculosis in the mouse, Lancet, 1950, *2*, 391.

(9) American Trudeau Society: The effect of cortisone and/or corticotropin on experimental tuberculous infection in animals: A statement prepared by the Laboratory Subcommittee on Medical Research, Am. Rev. Tuberc., 1952, *66*, 257.

VARIED DEGREES OF ISONIAZID RESISTANCE WITHIN STRAINS OF TUBERCLE BACILLI FROM SPUTUM AND PULMONARY CAVITIES[1,2]

SHEILA M. STEWART[3]

(Received for publication July 26, 1955)

INTRODUCTION

Pyle (1) and Mitchison (2) have shown that tubercle bacilli of varied degrees of streptomycin resistance can be present in any one sample of sputum. Variations in isoniazid resistance have been demonstrated by Tompsett (3). The present report deals with similar investigations of the isoniazid resistance of bacterial populations in sputum and cavities of patients with pulmonary tuberculosis who had been treated with isoniazid. Estimations of the percentage of the organisms resistant to the various concentrations of isoniazid were made directly on the material examined. These estimations have been termed isoniazid population studies.

METHOD

The method consisted essentially of inoculating a series of Löwenstein-Jensen plates containing varying concentrations of isoniazid with serial dilutions of the sputum concentrate. A plate which did not contain any drug was included in each series in order to give a total viable count. The numbers of organisms resistant to the various drug concentrations were thus determined. Owing to the tendency of *M. tuberculosis* to clump, the colony counts represent "viable units" rather than individual organisms.

Preparation of plates: Fourfold dilutions of isoniazid in sterile distilled water were added to Löwenstein-Jensen medium in the proportion of one part of dilution to 100 parts of medium to give final drug concentrations of 0.06 to 64.0 γ per ml. The isoniazid-egg medium mixture was then poured into 9.0 cm. Petri dishes, approximately 30 ml. being used for each plate. The plates were inspissated at temperatures between 75° and 80°C. in a hot air inspissator for half an hour on each of two successive days. Before use, the plates were dried in an incubator at 37°C. A disc of sterile filter paper was then placed in the lid of the Petri dish to prevent any water of condensation from dropping onto the surface of the medium.

Preparation of the sputum dilutions: A morning specimen of sputum, to which had been added an equal volume of 4 per cent sodium hydroxide (4) was mechanically shaken with glass beads for ten minutes at room temperature. The mixture was then kept at 37°C. for twenty to thirty minutes, depending on the nature of the sputum. It was subsequently centrifuged at 2,500 r.p.m. for thirty minutes. After the supernatant fluid had been removed and the deposit made neutral to phenol red with 8 per cent hydrochloric acid, approximately 25 ml. of sterile distilled water were added to the container and the mixture was re-centrifuged as before. The resulting concentrate was homogenized by shaking with 2 ml. of sterile distilled water in a mechanical shaker for ten minutes. A series of tenfold dilutions of the homogenate in sterile distilled water was then prepared, from which the plates were inoculated immediately.

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[2] This work was supported by a generous grant from the Royal Victoria Hospital Tuberculosis Trust.

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FIG. 1. Photograph showing the appearance of a plate of Löwenstein-Jensen medium after the inoculation with serial dilutions of a sputum concentrate and subsequent incubation at 37°C. for eight weeks. The highest concentration of the sputum concentrate was placed on the top left quadrant of the plate, the next on the top right, the next on the bottom left, and the lowest concentration on the bottom right quadrant.

The contents of lung cavities were treated in the same way as the sputum.

Inoculation of the plates: The plates were inoculated with Pasteur pipettes which delivered 50 drops to the ml. Two plates of each drug concentration and two control plates were inoculated from each specimen of sputum. One drop of one of four consecutive sputum dilutions was placed on the surface of each of the four quarters of every plate. The actual dilutions of the sputum concentrate used depended on the relative abundance of tubercle bacilli in the sputum. In most cases, dilutions of 1 in 10 to 1 in 10,000 were used for the control plates and the undiluted homogenate, and dilutions of 1 in 10 to 1 in 1,000 were used for the plates containing the drug. After inoculation the plates were left undisturbed at room temperature for twenty-four hours. The plates were then inverted, the filter papers removed, and paraffin wax was poured into the rim to prevent subsequent evaporation. The plates were incubated for eight weeks at 37°C. The photograph (figure 1) shows the appearance of a plate after eight weeks' incubation.

Recording of results: Counts were made from each plate at the lowest dilution of the sputum giving discrete colonies. The numbers of organisms resistant to the various drug concentrations were expressed as percentages of the total viable bacterial population.

Routine drug-susceptibility tests: Cultures were set up on Löwenstein-Jensen slopes from the neat sputum homogenate. All positive cultures were tested for isoniazid susceptibility in Löwenstein-Jensen medium at concentrations of 0.2, 1.0, 5.0, 10.0, and 50.0 γ per ml. (5).

RESULTS

Isolated Results on a Number of Patients

Studies on patients who had never received isoniazid: The results of investigations on sputum from patients who had never received isoniazid are given in table 1. It may be seen that in only one of the 8 cases (*Case 6*) was any growth found on the lowest concentration tested, i.e., 0.06 γ per ml. In that case only four colonies grew from 0.02 ml. of the undiluted sputum concentrate, i.e., only one in 4,000 of the organisms was resistant to 0.06 γ of the drug per ml. No growth

TABLE 1

THE RESULTS OF POPULATION STUDIES ON SPUTUM FROM PATIENTS WHO HAD NEVER RECEIVED ISONIAZID

Case Number	Total Viable Count per Ml. of Concentrate	Percentage of Tubercle Bacilli Resistant to Isoniazid γ *per ml.*						Isoniazid Resistance of Löwenstein-Jensen Culture*
		0.06	0.25	1.0	4.0	16.0	64.0	
1	12.5×10^5	0	0	0	0	0	0	Susceptible†
2	1.5×10^5	0	0	0	0	0	0	Susceptible
3	2.0×10^5	0	0	0	0	0	0	Susceptible
4	2.5×10^5	0	0	0	0	0	0	Susceptible
5	6.0×10^5	0	0	0	0	0	0	Susceptible
6	8.0×10^5	0.025	0	0	0	0	0	Susceptible
7	4.0×10^5	0	0	0	0	0	0	Susceptible
8	7.0×10^5	0	0	0	0	0	0	Susceptible

* By the method used in the Medical Research Council of Great Britain Trials of Isoniazid (5).

† Susceptible = fewer than 20 colonies on the slope containing 0.2 γ of isoniazid per ml. in Löwenstein-Jensen medium.

occurred at the higher concentrations. In all these cases the routine test showed the organisms to be susceptible to isoniazid concentrations of 0.2 γ per ml., the lowest concentration tested.

Studies on patients who had received isoniazid therapy and whose organisms were known to be resistant to the drug by the routine test: The results of the population studies on the sputum of 7 patients whose organisms were known to be resistant to isoniazid are shown in table 2. It will be noticed that in only one case (*Case 9*) were all of the organisms resistant to the highest concentration allowing growth. In many cases less than 1 per cent was resistant to the higher concentrations of the drug.

In the one case (*Case 9*) in which all of the tubercle bacilli appeared to be of the same degree of resistance, the organisms in the sputum were known to have been resistant to isoniazid for 516 days before the population study was carried out. The patient had not received any isoniazid for 471 days and, during this time, cultures from the sputum had shown a reduction in resistance from isoniazid concentrations of 50.0 γ per ml. to concentrations of 0.2 to 1.0 γ per ml. This low degree of resistance was maintained for eight months before the examination of the sputum. The population study showed all of the organisms in the sputum to be resistant to isoniazid concentrations of 4.0 γ per ml.

In 2 cases (*Cases 10* and *11*) the cultures from the sputum were resistant to 10.0 and 50.0 γ per ml., respectively, at the time of the population study. In each case, at least one-third of the tubercle bacilli was shown to be resistant to isoniazid concentrations of 16.0 γ per ml. These patients had been given isoniazid for 353 days and two years, respectively, and they were both receiving the drug at the time the microbial population study was carried out.

Population studies on the sputum from the remaining 4 patients all showed a marked variation in isoniazid resistance. Two of these (*Cases 12* and *13*) had

TABLE 2

THE RESULTS OF POPULATION STUDIES ON SPUTUM FROM PATIENTS WHO HAD RECEIVED THE DRUG AND WHOSE ORGANISMS WERE KNOWN TO BE RESISTANT BY THE ROUTINE DRUG TEST

Case Number	Duration of Isoniazid Therapy	Post-Treatment Interval Before Population Study	Duration of Resistance Before Population Study	Viable Count of Concentrate per Ml.	Percentage of Organisms Resistant to Isoniazid γ per ml.						Routine Resistance Test*
					0.06	0.25	1.0	4.0	16.0	64.0	
	days	*days*	*days*								*γ per ml.*
9	196	471	516	5.5×10^5	100	100.00	—	100.00	0	0	1.0
10	353	0	271	3.5×10^6	—	100.00	100.0	100.00	36.00	0	10.0
11	Approximately 750	0	Not known	8.5×10^5	100	100.00	100.0	100.00	33.00	4.10	50.0
12	180	420	Not known	3.5×10^5	29	36.00	1.3	0.42	0.16	0.04	50.0
13	42	264	Not known	3.5×10^5	86	57.00	11.0	1.40	0	0	5.0
14	328†	0	350	5.5×10^6	100	60.00	8.0	0.13	0	0	1.0
15	144	0	82	2.0×10^6	50	0.75	0.4	0.50	0.75	0.37	50.0

* By the method used in the Medical Research Council of Great Britain Trials of Isoniazid (5); cultures tested on Löwenstein-Jensen medium containing 0.2, 1.0, 5.0, 10.0, and 50.0 γ of isoniazid per ml.

† This patient received two courses of isoniazid of 168 and 160 days' duration, respectively, with a break of 63 days between.

received no isoniazid for 264 and 420 days, respectively. Frequent resistance tests had not been carried out on cultures from the sputum of these patients. In the third patient (*Case 14*) the organisms from the sputum had shown a reduction in degree of isoniazid resistance during the period in which the patient was not receiving the drug; during a second course of isoniazid the results on the sputum cultures varied considerably. The fourth patient (*Case 15*) had received continuous isoniazid therapy combined with oxytetracycline, 2 gm. daily, for 144 days before the population study was done. Sputum cultures before the population study had shown a low degree of resistance, but on later readings an increase was found.

The results of the routine resistance tests agreed well with the results of the population studies.

Weekly Investigations on Two Patients

It is known from the weekly routine testing of the isoniazid resistance of tubercle bacilli isolated from patients who received the drug alone that in most cases a high degree of resistance develops rapidly after the first appearance of resistant organisms (6). In an attempt to determine whether, with this rapid development of resistance, all of the organisms were affected simultaneously or whether only a few became highly resistant, weekly population studies were carried out on 2 patients.

By the time this work was begun it was already known that bacterial resistance

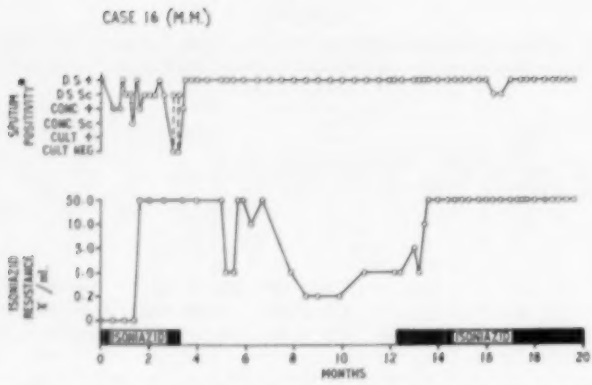

FIG. 2. Chart showing the approximate numbers of tubercle bacilli in the sputum and their isoniazid resistance during the first and second courses of isoniazid treatment of a patient (*Case 16*) on whom weekly population studies were made during the second course of isoniazid.

* DS + = Direct smear and culture positive for tubercle bacilli
DS Sc. = Direct smear with scanty number of bacilli (positive after more than five minutes' search) and culture positive
Conc. + = Concentrate film and culture positive
Conc. Sc. = Concentrate smear with scanty number of bacilli (positive after more than five minutes' search) and culture positive
Cult. + = Direct and concentrate films negative; culture positive
Cult. neg. = Direct and concentrate films and culture negative
Dotted lines indicate a positive smear with negative culture

to the drug would develop in a large proportion of patients treated with isoniazid alone (7–9). It was therefore not the usual practice to use such treatment if the patient's organisms were still susceptible to the drug. Nevertheless, there were 2 patients in the present series who had been treated with isoniazid alone in the original Medical Research Council of Great Britain Trials of Isoniazid and whose organisms were drug resistant. In neither case was any definitive therapy possible. Treatment had been stopped after the organisms had been found to be resistant to isoniazid concentrations of 50.0 γ per ml. by the routine test. During the subsequent months the tubercle bacilli from the sputum of both patients showed a marked decrease in isoniazid resistance. By the seventh month after stopping treatment in one case (*Case 16*) and the ninth month in the other (*Case 17*), the organisms were resistant only to considerably lower concentrations of isoniazid, i.e., 0.2 to 1.0 γ per ml. It was therefore decided to re-treat these patients with isoniazid alone in a dose of 100 mg. twice daily and to follow the changes that occurred in the resistance of their tubercle bacilli.

Case 16: The approximate number of tubercle bacilli in the sputum of the first patient and the results of the routine isoniazid-susceptibility tests are shown in figure 2. It will be seen that during the first course of isoniazid therapy there was some reduction in the population of tubercle bacilli in the sputum. Six weeks

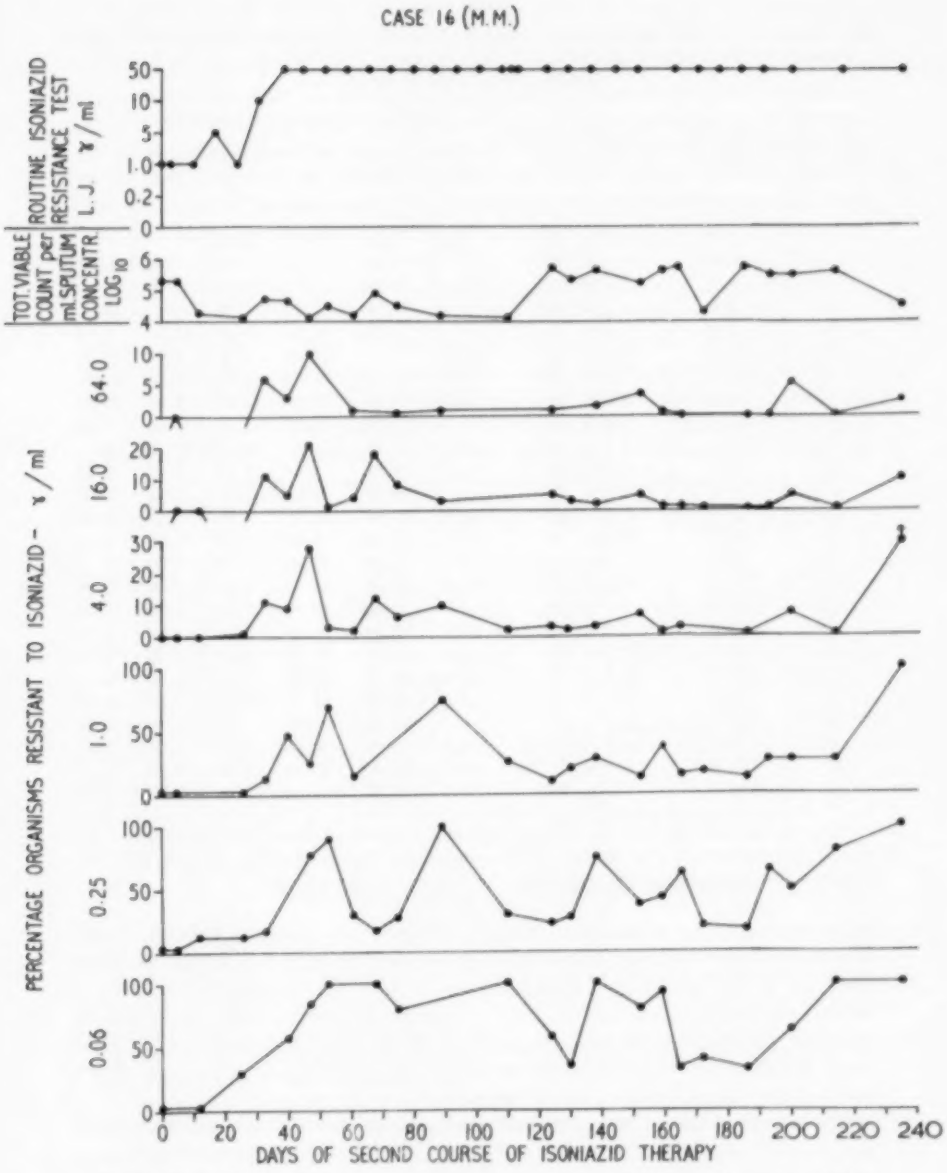

FIG. 3. Chart showing the results of the weekly population studies on the sputum during the second course of isoniazid treatment, together with the results of the routine resistance tests over this period (*Case 16*).

TABLE 3

THE RESULTS OF THE WEEKLY POPULATION STUDIES OF THE SPUTUM DURING THE SECOND COURSE OF ISONIAZID TREATMENT, TOGETHER WITH THE RESULTS OF THE ROUTINE RESISTANCE TESTS (*Case 16*)

Days After Starting Re-Treatment with Isoniazid	Isoniazid Resistance of Löwenstein-Jensen Culture*	Total Viable Count per Ml. of Sputum Concentrate	Percentage of Organisms Resistant to Isoniazid γ *per ml.*					
			0.06	0.25	1.0	4.0	16.0	64.0
	γ *per ml.*							
0	1.0	2.9×10^5	3.8	0.38	0.07	0.02	0	0
5	1.0	3.0×10^5	—	0.67	0.50	0.07	0.05	0.01
12	1.0	2.5×10^4	4.8	12.00	—	0.20	0.20	0
26	5.0	1.5×10^4	24.0	12.00	1.70	1.40	0	0
33	1.0	7.5×10^4	—	17.00	13.00	11.00	11.00	6.00
40	10.0	7.5×10^3	57.0	—	46.00	9.30	4.70	3.10
47	50.0	1.8×10^4	86.0	78.00	25.00	28.00	21.00	10.00
53	50.0	5.0×10^4	100.0	90.00	70.00	3.00	1.00	0
61	50.0	2.0×10^4	—	30.00	15.00	2.00	4.00	1.00
68	50.0	9.0×10^4	100.0	17.00	—	12.00	18.00	0
75	50.0	5.0×10^4	80.0	28.00	—	6.00	8.50	0.45
89	50.0	2.0×10^5	—	100.00	75.00	10.00	2.50	1.00
110	50.0	0.7×10^5	100.0	30.00	23.00	2.00	—	11.00
124	50.0	7.0×10^5	57.0	23.00	8.00	3.30	5.00	1.00
130	50.0	3.3×10^5	35.0	28.00	17.00	2.00	2.00	0
138	50.0	6.0×10^5	100.0	75.00	29.00	2.50	2.50	1.80
152	50.0	2.0×10^5	80.0	37.50	12.50	7.50	5.70	3.70
159	50.0	6.0×10^5	92.0	42.00	38.00	1.70	1.50	0.33
165	50.0	7.5×10^5	28.0	64.00	15.00	2.70	1.30	0.02
172	50.0	2.5×10^5	40.0	20.00	18.00	—	1.00	0
186	50.0	7.0×10^5	27.0	19.00	12.00	0.30	0.60	0.01
193	50.0	2.5×10^5	—	64.00	28.00	—	1.10	0.16
200	50.0	2.7×10^5	63.0	50.00	27.00	7.30	5.50	5.50
214	50.0	5.5×10^5	100.0	82.00	27.00	0.55	0.10	0.01
235	50.0	5.0×10^5	100.0	100.00	100.00	75.00	10.00	2.80

* By the method used in the Medical Research Council of Great Britain Trials of Isoniazid (5); cultures tested on Löwenstein-Jensen medium containing 0.2, 1.0, 5.0, 10.0, and 50.0 γ of isoniazid per ml.

after the beginning of isoniazid therapy, the patient's strain of tubercle bacilli was found to be resistant to isoniazid concentrations of 50.0 γ per ml. A disappearance of the more highly drug-resistant bacilli from the sputum occurred in the subsequent five months but, with a second course of isoniazid, there was no effect on the discharge of tubercle bacilli in the sputum. Moreover, by the sixth week of treatment, tubercle bacilli resistant to isoniazid concentrations of 50.0 γ per ml. were again present in the sputum.

The results of the weekly microbial population studies of this patient's sputum are shown in figure 3, along with the results of the routine drug-resistance tests over this period. The actual percentages of the organisms resistant to the various concentrations of isoniazid are shown in table 3. At the beginning of re-treatment, only 3.8 per cent of the tubercle bacilli were resistant to 0.06 γ of isoni-

azid per ml., and less than 0.1 per cent to concentrations of 1.0 γ per ml. or higher. By the fifty-third day of re-treatment, 100 per cent of the organisms were resistant to 0.06 γ per ml. About this time an increase also occurred in the number of organisms resistant to the higher concentrations, but at no time were 100 per cent of this patient's strain resistant to isoniazid concentrations of 4.0 γ per ml. or higher. Indeed, the percentage of the population which was resistant to 16.0 γ per ml. was never greater than 21.0 and the percentage resistant to 64.0 γ per ml. was never greater than 11.0 in spite of continuous treatment for 235 days.

Examination of 17 individual lesions obtained at post-mortem examination of this patient twenty-one weeks after the end of the second course of treatment by methods described elsewhere (10) showed a marked variation in the degrees of resistance of the tubercle bacilli cultured from the various sites.

Case 17: In figure 4 may be seen the approximate numbers of tubercle bacilli in the sputum and the resistance studies made of the strain from the second patient who was studied at weekly intervals. There was some reduction in the number of tubercle bacilli in the sputum during the first course of isoniazid until resistant organisms appeared in approximately the sixth week. A more pronounced reduction in the number of tubercle bacilli discharged in the sputum occurred during a short course of streptomycin and sodium *p*-aminosalicylate

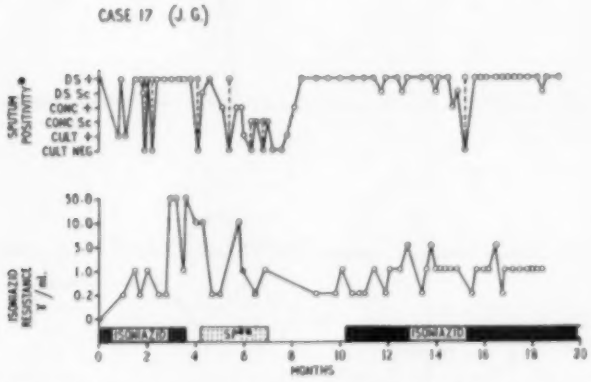

FIG. 4. Chart showing the sputum positivity and the isoniazid resistance of the tubercle bacilli during the first and second courses of isoniazid treatment of a patient (*Case 17*) on whom weekly population studies were carried out during the second course of isoniazid.

* DS + = Direct smear and culture positive
DS Sc. = Direct smear with scanty number of bacilli (positive after more than five minutes' search) and culture positive
Conc. + = Concentrate film and culture positive
Conc. Sc. = Concentrate film with scanty number of bacilli (positive after more than five minutes' search) and culture positive
Cult. + = Direct and concentrate films negative; culture positive
Cult. neg. = Direct and concentrate films and culture negative.
Dotted lines indicate a positive smear with a negative culture
** SP = Streptomycin, 1 gm. daily, with PAS, 20 gm. daily.

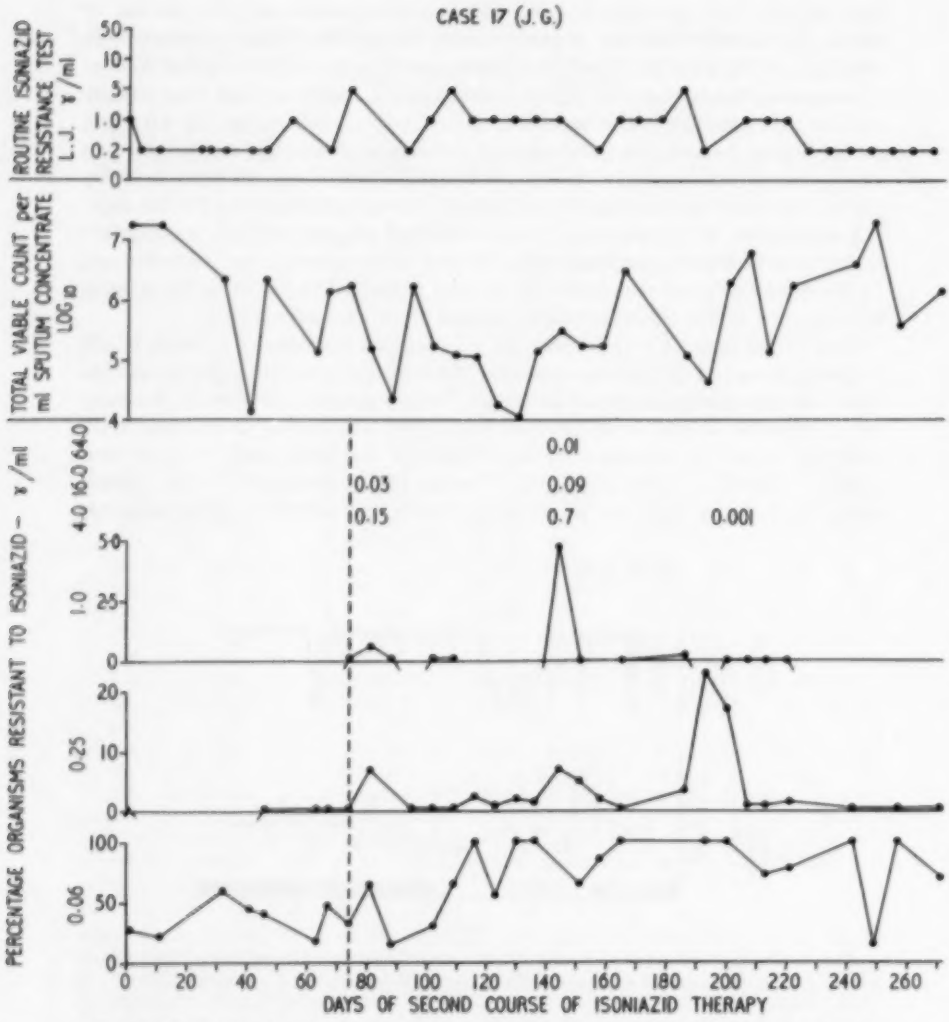

FIG. 5. Chart showing the results of the weekly population studies on the sputum of a patient (*Case 17*) during the second course of isoniazid treatment. On specimens to the left of the vertical dotted line the undiluted sputum concentrate was not inoculated onto any of the plates.

(PAS), to which drugs the patient's strain was susceptible. At this time the prognosis in this case was considered hopeless. Accordingly, streptomycin-PAS treatment was stopped after three months and the number of tubercle bacilli discharged in the sputum showed a considerable increase. The second course of isoniazid had no effect on the number of bacilli in the sputum and had only slight

effect on the level of isoniazid resistance of the strain. At no time were bacilli resistant to isoniazid concentrations of 50.0 γ per ml. observed in contrast to the situation during the first course of isoniazid therapy.

The results of the weekly population studies on the sputum from this patient may be seen in figure 5, and the detailed results are presented in table 4. It will be noticed that only 25 per cent of the organisms were resistant to isoniazid

TABLE 4

THE RESULTS OF THE WEEKLY POPULATION STUDIES ON THE SPUTUM DURING THE SECOND COURSE OF ISONIAZID TREATMENT (*Case 17*)

(On specimens above the dotted line the undiluted sputum concentrate was not inoculated onto any of the plates)

Days After Starting Re-Treatment with Isoniazid	Isoniazid Resistance of Löwenstein-Jensen Culture*	Total Viable Count per Ml. of Sputum Concentrate	Percentage of Organisms Resistant to Isoniazid γ *per ml.*					
			0.06	0.25	1.0	4.0	16.0	64.0
	γ *per ml.*							
0	0.2	3.5×10^7	25	0.06	0	0	0	0
11	0.2	2.7×10^7	22	0	0	0	0	0
32	0.2	3.4×10^6	60	0	0	0	0	0
41	1.0	1.6×10^6	44	0	0	0	0	0
46	0.2	3.3×10^6	42	0.15	0	0	0	0
63	1.0	1.4×10^5	18	0.36	0	0	0	0
67	1.0	1.9×10^6	47	0.13	0	0	0	0
74	1.0	2.2×10^6	32	0.25	0.005	0	0	0
81	5.0	1.9×10^5	65	7.00	0.650	0.150	0.03	0
88	0.2	3.3×10^5	14	—	0.150	0	0	0
95	1.0	2.3×10^6	—	0.74	0	0	0	0
102	5.0	2.5×10^5	30	0.35	0.090	0	0	0
109	1.0	1.5×10^5	65	0.70	0.170	0	0	0
116	1.0	0.7×10^5	100	2.60	0	0	0	0
123	1.0	2.8×10^5	56	0.90	0	0	0	0
130	1.0	0.5×10^5	100	2.00	0	0	0	0
136	1.0	1.5×10^5	100	1.60	0	0	0	0
144	1.0	5.0×10^5	—	7.00	4.600	0.700	0.09	0.01
151	0.2	2.5×10^5	65	5.10	0.020	0	0	0
158	1.0	2.4×10^5	84	2.10	0	0	0	0
165	1.0	5.0×10^6	100	0.55	0.004	0	0	0
186	1.0	1.0×10^5	—	3.75	0.275	0	0	0
193	1.0	6.5×10^5	100	23.00	0	0	0	0
200	5.0	3.6×10^6	100	17.00	0.050	0.001	0	0
207	0.2	8.0×10^6	—	0.80	0.001	0	0	0
213	1.0	1.3×10^5	72	0.88	0.840	0	0	0
221	1.0	2.6×10^6	77	1.50	0.030	0	0	0
242	1.0	6.3×10^6	100	0.72	0	0	0	0
249	1.0	3.1×10^7	11	0.02	0	0	0	0
257	1.0	6.0×10^5	100	0.63	0	0	0	0
271	1.0	1.2×10^6	70	0.15	0	0	0	0

* By the method used in the Medical Research Council of Great Britain Trials of Isoniazid (5); cultures tested on Löwenstein-Jensen medium containing 0.2, 1.0, 5.0, 10.0, and 50.0 γ of isoniazid per ml.

TABLE 5

THE RESULTS OF POPULATION STUDIES ON THE CONTENTS OF LUNG CAVITIES

(In one case (*Case 18*) the lung specimen was obtained at autopsy and in the other two at operation)

Case Number	Duration of Isoniazid Therapy	Post-Treatment Interval Before Population Study	Duration of Resistance Before Population Study	Viable Count of Concentrate per Ml.	Percentage of Organisms Resistant to Isoniazid γ per ml.						Resistance Test from Löwenstein-Jensen Culture*
					0.06	0.25	1.0	4.0	16.0	64.0	
	days	*days*	*days*								γ *per ml.*
18	154	209	316	6.5×10^6	—	100	100	11	1.7	0	10.0
19a	152	512	Not known	5.0×10^5	100	100	100	100	50.0	36.0	50.0
19b				3.0×10^5	100	100	100	25	13.0	6.7	50.0
20	236	8	86	5.0×10^4	100	100	18	0	0	0	2.5

* By the method used in the Medical Research Council of Great Britain Trials of Isoniazid (5); cultures tested on Löwenstein-Jensen medium containing 0.2, 0.5, 1.0, 2.5, 5.0, 10.0, 25.0, and 50.0 γ of isoniazid per ml.

concentrations of 0.06 γ per ml. before the beginning of the second course of isoniazid. It was not until approximately the one hundredth day after the reinstitution of isoniazid therapy that 100 per cent of the organisms were found to be resistant to 0.06 γ per ml. About this time there was a tendency for the results of the routine tests of this patient's organisms to rise from 0.2 γ per ml. to 1.0 γ per ml. The undiluted sputum homogenates were not used in these observations until the seventy-fourth re-treatment day. Organisms resistant to isoniazid concentrations of 0.25 γ per ml. and higher may have been present before this time but in such small numbers that they were not detected. At no time during the 270 days of re-treatment did the percentage of organisms resistant to 0.25 γ per ml. rise above 23 per cent, and this value was generally below 10 per cent. The number of organisms resistant to 1.0 γ per ml. was extremely low; at the higher concentrations of isoniazid, only a very few resistant organisms occurred, and those very infrequently.

Studies on the Contents of Lung Cavities

The results of the investigations on the contents of lung cavities from 3 patients whose organisms were known to be isoniazid resistant are shown in table 5. It will be noted that, in the cavity of the patient whose isoniazid therapy was discontinued only eight days before the population study was carried out, the resistance of the organisms was more uniform than in the other 2 patients. It should be noted, however, that this patient had received a longer course of isoniazid than had the other 2 patients. In no case were 100 per cent of the organisms resistant to the highest isoniazid concentration at which growth occurred.

DISCUSSION

Pyle (1) and Mitchison (2) have shown that organisms with varying degrees of resistance to streptomycin can be found in a single specimen of sputum from

a patient whose organisms are resistant to the drug. Tompsett (3) has demonstrated a similar variation with regard to isoniazid. His investigations were carried out on liquid medium cultures from the sputum of patients who were receiving the drug alone for the first time. He found that in many cases only a low percentage of the organisms were resistant to the higher concentrations of the drug in spite of continuous treatment for seven months. The results in the present series using undiluted sputum confirm these earlier results.

It appears that the proportion of highly resistant bacilli found in the sputum of patients with isoniazid-resistant organisms may be rather lower than is the case with streptomycin. In only one case (*Case 9*) in the present series were all of the organisms resistant to the highest concentration of the drug allowing growth (tables 2–4). In none of the contents of lung cavities was uniformity of resistance found (table 5), although the degree of variation among the individual bacilli in these lesions was less marked than among the bacilli in the sputum of other patients (table 2).

In 2 cases in the present study (*Cases 10* and *11*) all of the organisms were highly resistant to isoniazid. The patients had received the drug for 353 days and two years, respectively, and in both cases the organisms in the sputum were known to be highly resistant. It seems likely that in these cases the prolonged treatment had resulted in the development of a high degree of resistance throughout the lung.

In one case (*Case 9*) the organisms were all equally resistant, although not to a high degree. The organisms from the sputum in this patient were known to have been resistant to isoniazid concentrations of 50 γ per ml. during treatment. After the drug was stopped, however, a marked reduction occurred and, subsequently, the tubercle bacilli in the sputum were shown repeatedly to be resistant to 1.0 to 0.2 γ per ml. The uniformity of the resistance of the organisms in the sputum at the time of the population study suggests that a reduction in the number of bacilli resistant to high concentrations of the drug may have occurred throughout the lungs as has been shown to occur by the examination of individual lung lesions (10). Alternatively, the drainage from the lesions containing highly resistant bacilli may merely have become blocked.

An increase in the degree of isoniazid resistance of the organisms in the sputum was known to have occurred after the population study was done in one case (*Case 15*). The variation in the degree of isoniazid resistance of the organisms in the sputum may have been due to the fact that all of the organisms in the lung had not yet reached their maximum resistance (10).

Three patients (*Cases 14*, *16*, and *17*) received two courses of isoniazid. In each case, cultures from the sputum were highly resistant by the end of the first course of treatment, but there was a marked reduction in the degree of resistance during the time when no isoniazid was being given. In 2 cases (*Cases 14* and *16*), with the second course of isoniazid, highly resistant organisms were again isolated. In the third patient (*Case 17*), however, the organisms showed only a moderate degree of isoniazid resistance. In none of these 3 cases did all

of the organisms become resistant to the maximum concentration of isoniazid allowing growth in spite of second courses of the drug of 160, 235, and 270 days, respectively. In one of these patients (*Case 16*) the varied degree of resistance of the organisms in the sputum was probably a reflection of the marked variation of resistance of the tubercle bacilli from different lesions in the lung. The second courses of isoniazid treatment were shorter than either of the initial courses given to the 2 patients (*Cases 10* and *11*) in whom all of the organisms became highly resistant. Had the isoniazid been continued for a longer time it is possible that a greater uniformity in the degree of isoniazid resistance might have developed.

In the remaining 2 patients, resistance tests on the cultures from the sputum were obtained too infrequently to correlate them with the results of the population studies.

Canetti and Sáenz (11) have shown that tubercle bacilli of varied degrees of streptomycin resistance may be isolated from different lesions within the same lung. Recent investigations in this unit have shown that a similar wide variation in isoniazid resistance can occur (10). It is therefore reasonable to suppose that sputum derived simultaneously from various sites may contain organisms of various degrees of resistance and that the proportion of the organisms resistant to the different concentrations of the drug may depend on the sources of the sputum. This may account for the marked fluctuations seen in the results shown in tables 3 and 4 and may also explain the variations which sometimes occur in a series of routine tests for isoniazid resistance on consecutive cultures from the same patient.

The clinical improvement which may occur in a patient on isoniazid therapy after the appearance of isoniazid-resistant tubercle bacilli in the sputum may be due to the greater ability of the patient's defenses to deal with the much reduced bacterial population. The results in *Cases 12*, *15*, *16*, and *17*, however, suggest that for a time the improvement may be due in part to the presence of a high percentage of isoniazid-susceptible organisms although the patient's strain appears to be resistant by a routine test.

The detailed studies on the 2 patients who were receiving a second course of isoniazid therapy gave similar results to those found by Tompsett (3) during first courses of the drug. Therefore, it may be that increases in the degree of resistance which occur with re-treatment with the drug after apparent reversion follow the same pattern as in the original development of resistance. The low percentage of organisms resistant to the higher concentrations of the drug and the similarity of these results to those found by Mitchison (2) with streptomycin-resistant organisms are consistent with the suggestion that isoniazid resistance may develop by "jump" mutations, as Bryson and Demerec (12) postulated for streptomycin.

There is a close correlation between the results of the bacterial population studies and the routine resistance tests on cultures from the same sputum (table 2). It would appear that the routine test will detect as few as 0.1 per cent of resistant organisms present in the sputum. This may be compared with a

figure of 3 per cent given by Mitchison (2) as the lowest percentage of streptomycin-resistant organisms in a liquid medium culture detectable by the routine liquid medium test. The close correlation between the highest isoniazid concentration allowing growth in the population studies and that allowing growth on the routine test would suggest that, during the normal incubation period of a primary culture of tubercle bacilli from sputum, there is no appreciable loss of isoniazid resistance.

In conclusion, these investigations show that there is usually a marked variation in the degree of isoniazid resistance in the bacilli present in the sputum of patients whose organisms have become resistant under treatment. Although this variation is also present in the organisms from individual lung lesions, it is not so marked as that in the tubercle bacilli discharged in the sputum.

SUMMARY

The percentage of tubercle bacilli in the sputum resistant to various concentrations of isoniazid was determined in 8 patients who had never received the drug. Not more than one in 4,000 of the organisms were resistant to 0.06 γ isoniazid per ml. of Löwenstein-Jensen medium.

In 5 of the 7 patients whose organisms were known to be resistant, a marked variation in the degree of resistance of individual organisms was found.

Weekly investigations were carried out during a second course of isoniazid in 2 patients whose organisms had become resistant during isoniazid therapy but had shown a reduction in the degree of isoniazid resistance following cessation of the therapy. In neither case did all of the organisms show the same degree of resistance in spite of re-treatment with isoniazid for 235 and 270 days, respectively.

Variation in the degree of isoniazid resistance among individual tubercle bacilli was present in the contents of lung cavities although the phenomenon was observed to occur to a lesser extent than in sputum.

A close correlation was found between the results of the bacterial population studies and the routine resistance tests.

SUMARIO

Grados Variables de Resistencia a la Isoniacida en Cepas de Bacilos Tuberculosos Procedentes del Esputo y de Cavernas Pulmonares

En 8 enfermos que jamás habían recibido isoniacida, se determinó en el esputo el porcentaje de microbios resistentes a varias concentraciones de la droga. No hubo más de uno en 4,000 que fuera resistente a 0.06 γ de isoniacida por ml. de medio de Löwenstein-Jensen.

En 5 de los 7 enfermos cuyos microbios se sabía que eran resistentes, observóse una notable heterogeneidad en la intensidad de la resistencia.

Durante una segunda serie de isoniacida, lleváronse a cabo investigaciones semanales en 2 enfermos cuyos gérmenes se habían vuelto resistentes durante la primera serie, pero que habían mostrado después disminución en la intensidad de su resistencia a la isoniacida. Ni en uno ni en otro caso, mostraron todos los microbios el mismo grado de resistencia a pesar de una terapéutica continua durante 235 y 270 días, respectivamente.

Notóse también heterogeneidad de la resistencia en el contenido de las cavernas pulmonares, aunque en menor grado que en el esputo.

Observóse notable correlación entre los resultados de los estudios de la población bacteriana y las pruebas habituales de la resistencia.

RESUME

Les divers degrés de la résistance a l'isoniazide dans des souches de bacilles tuberculeux extraits de crachats et de cavernes pulmonaires

Le pourcentage de germes extraits de crachats qui présentaient une résistance à diverses concentrations d'isoniazide a été déterminé chez 8 malades n'ayant jamais reçu l'antibiotique. Parmi 4.000 de ces bacilles, un seul présentait une résistance à 0,06 γ d'isoniazide par ml. du milieu de Löwenstein-Jensen.

Chez 5 des 7 patients porteurs de germes reconnus résistants, une hétérogénéité marquée dans le degré de résistance a été observée. Des investigations hebdomadaires ont été poursuivies durant une seconde cure d'isoniazide chez deux patients dont les bacilles avaient acquis la résistance au cours de la première cure, mais qui avaient ultérieurement présenté une réduction de degré de l'isoniazide-résistance. En dépit d'un traitement continu de 235 et 270 jours, respectivement, les germes ne présentaient pas tous le même degré de résistance.

L'hétérogénéité de la résistance existait dans le contenu des excavations pulmonaires, mais elle était moins marquée que dans l'expectoration.

Une étroite corrélation a été observée entre les résultats des études sur la population bactérienne et les tests de résistance classiques.

Acknowledgments

The writer would like to thank Professor J. W. Crofton for his help and advice in this work; Drs. I. W. B. Grant, N. W. Horne, and W. A. Murray for allowing me to investigate their cases; Mr. A. Logan for giving me access to the surgical specimens; and Dr. A. T. Wallace who carried out many of the routine tests. She is grateful to Mr. D. Hay for technical assistance; and to the nursing staff of the City Hospital, Edinburgh, for their help.

REFERENCES

(1) Pyle, M. M.: Relative numbers of resistant tubercle bacilli in sputa of patients before and during treatment with streptomycin, Proc. Staff Meet., Mayo Clin., 1947, *22*, 465.

(2) Mitchison, D. A.: Development of streptomycin-resistant strains of tubercle bacilli in pulmonary tuberculosis, Thorax, 1950, *5*, 144.

(3) Tompsett, R.: Quantitative observations on the pattern of emergence of resistance to isoniazid, Am. Rev. Tubere., 1954, *70*, 91.

(4) Petroff, S. A.: A new and rapid method for the isolation and cultivation of tubercle bacilli directly from sputum and feces, J. Exper. Med., 1915, *21*, 38.

(5) Medical Research Council: Laboratory techniques for the determination of sensitivity of tubercle bacilli to isoniazid, streptomycin and P.A.S., Lancet, 1953, *2*, 213.

(6) Wallace, A. T., Stewart, S. M., Turnbull, F. A. W., and Crofton, J. W.: Isoniazid resistance in patients with pulmonary tuberculosis treated on isoniazid alone, Tubercle, 1954, *35*, 164.

(7) Dye, W. E., Lynch, H. P., and Brees, A. G.: Incidence of bacterial resistance encountered with tuberculosis chemotherapy regimens employing isoniazid alone and in combination with intermittent streptomycin, Am. Rev. Tubere., 1953, *67*, 106.

(8) Medical Research Council: The treatment of pulmonary tuberculosis with isoniazid, Brit. M. J., 1952, No. 4787, 735.

(9) Medical Research Council: Isoniazid in the treatment of pulmonary tuberculosis, Brit. M. J., 1953, No. 4809, 521.

(10) Turnbull, F. W. A., and Stewart, S. M.: Studies on the distribution of drug-resistant tubercle bacilli within the lung, Am. Rev. Tuberc., 1956, *73*, 406.

(11) Canetti, G., and Sáenz, A.: La streptomycino-résistance de souches de bacilles de Koch provenant de lésions pulmonaires différentes d'un même malade, Ann. Inst. Pasteur, 1951, *80*, 238.

(12) Bryson, V., and Demerec, M.: Patterns of resistance of antimicrobial agents, Ann. New York Acad. Sc., 1950, *53*, 283.

STUDIES ON THE DISTRIBUTION OF DRUG-RESISTANT TUBERCLE BACILLI WITHIN THE LUNG[1,2]

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(Received for publication July 26, 1955)

INTRODUCTION

When bacterial resistance to streptomycin or to isoniazid occurs in cases of pulmonary tuberculosis, organisms of various degrees of resistance may be isolated from a single sample of sputum (1–3). Variations may also be found in the degree of resistance of tubercle bacilli from different lesions within the same lung (3–8). The present report deals with a detailed study of the distribution of streptomycin- and isoniazid-resistant tubercle bacilli in specimens of lung tissue.

MATERIALS AND METHODS

Lung tissue was examined from 13 patients whose organisms were known to be resistant to both streptomycin and isoniazid. These patients had been treated with these drugs on regimens which would not now be used, as they are known to be ineffective in preventing the appearance of drug-resistant organisms. Isoniazid-resistance tests were carried out in all 13 cases, but streptomycin-resistance tests were only done in 8 of them. Eight patients (*Cases 1, 4, 5, 6, 7, 9, 11,* and *12*) were examined post mortem, and specimens of lung tissue were obtained from the other 5 patients (*Cases 2, 3, 8, 10,* and *13*) at operation. All cases were classified as far advanced pulmonary tuberculosis with cavitation.

Sampling of lesions: The specimens were examined within six hours of the operation or within twenty-four hours of the patient's death, except in one case (*Case 11*) in which post-mortem examination was delayed for seventy-two hours. The main sites of the disease were located by means of a recent roentgenogram and samples were taken from each of these. In addition, a number of lesions were sampled which were not visible on the roentgenogram. Precautions were taken to minimize the spread of tubercle bacilli from one site of infection to another. A small incision was made through the wall of the cavities and the contents were removed. Caseous foci were cut out and macerated with scissors.

Isolation of tubercle bacilli: The specimens were suspended in a small volume of sterile distilled water and an equal volume of 4 per cent sodium hydroxide was added. After the addition of glass beads, the mixture was shaken mechanically for ten minutes and then placed at 37°C. for twenty minutes. After centrifuging at 2,500 r.p.m. for twenty minutes, the supernatant fluid was poured off and the deposit was made neutral to phenol red with 8 per cent hydrochloric acid. Twenty-five milliliters of sterile distilled water were added and the material was centrifuged as before. The supernatant fluid was poured off. A Ziehl-Neelsen film was made from part of the deposit and the remainder was inoculated onto three Löwenstein-Jensen slopes by means of a Pasteur pipette. Cultures were incubated at 37°C. They were examined weekly and only discarded as negative after twelve weeks' incubation. Before the addition of the alkali, the aqueous suspensions of some of the specimens were injected into guinea pigs. All of the animals were killed twelve weeks after inoculation and cultures were made from the lesions.

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[2] This work was supported by a generous grant from the Royal Victoria Hospital Tuberculosis Trust.

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Bacterial drug-resistance tests: In all cases, positive cultures were tested for resistance to isoniazid and in 8 cases streptomycin-resistance tests were also done. When all three cultures from a lesion were positive, the slope with the heaviest growth was used. When primary cultures were negative but guinea pig inoculation was positive, resistance tests were carried out on the tubercle bacilli isolated from the animal organs.

The streptomycin-resistance tests were done by the method recommended by the Medical Research Council of Great Britain (9), using the liquid medium of Davis and Dubos. The actual concentrations of streptomycin added to the medium were 128, 32, and 8 γ, and then, by twofold dilutions, to 0.125 γ per ml. In 2 of the cases, the organisms from the sputum were found to be susceptible by this test, but resistant when tested on Löwenstein-Jensen medium by a method already reported (10). Therefore, in these cases both liquid and solid medium drug-susceptibility tests were done on the cultures from the lung lesions. The actual concentrations of streptomycin added to the solid medium were 64.0 to 1.0 γ per ml. by twofold dilutions. The results of both methods were expressed as resistance ratios, that is, the ratio of the minimum inhibitory concentration for the test organism to the minimum inhibitory concentration for the standard H37Rv strain of *M. tuberculosis*.

The method for detecting isoniazid resistance was that recommended by the Medical Research Council of Great Britain (9), using Löwenstein-Jensen medium. The isoniazid concentrations were 50.0, 25.0, 10.0, 5.0, 2.5, 1.0, 0.5, and 0.2 γ per ml. The results were expressed as the highest concentration allowing the growth of 20 or more colonies.

Streptomycin- and isoniazid-resistance tests were also done on the last culture of tubercle bacilli isolated from the sputum before examination of the lungs. For the sputum cultures, the range of streptomycin concentrations used was 8.0 to 0.125 γ per ml. by twofold dilutions. The isoniazid resistance was only tested at 50.0, 10.0, 5.0, 1.0, and 0.2 γ per ml.

Cultures were considered to be resistant to streptomycin when a resistance ratio of 4 or more was obtained. This means that growth was inhibited by at least 1.0 γ per ml. of liquid medium, or at least 16.0 γ per ml. of solid medium. A resistance ratio of 4 by either method has been shown to be outside the normal range at the 1 per cent level (10). Clinical evidence of the significance of this degree of resistance will be published elsewhere. A growth of 20 or more colonies on the slope containing 0.2 γ of isoniazid per ml. was taken as indicating "doubtful" resistance, and a similar growth on 1.0 γ per ml. as "definite" resistance (9).

RESULTS

Streptomycin

Resistance of tubercle bacilli from the lung lesions: In table 1 may be seen the results for 8 patients of streptomycin-resistance tests in liquid medium on cultures from the lung lesions and from the last available culture of the sputum. Details are also given of the courses of chemotherapy, the times since streptomycin was last administered, and the duration of streptomycin resistance before the examination of the lung.

Owing to the experimental errors of the resistance test, cultures from the lesions were considered to show a significant variation in the degree of streptomycin resistance only if there was at least a fourfold difference between the results. This difference between the maximum and minimum degrees of resistance of the lung cultures was present in 6 cases. The resistance ratios varied from 64 to greater than 256 in one case (*Case 10*); from 1 to greater than 256 in another (*Case 11*); from 1 to 16 in a third (*Case 3*); and from 1 to 8 in a fourth (*Case 7*). In 2 cases (*Cases 1* and *4*) the readings varied from 16 to 256. In the remaining 2 cases (*Cases 5* and *6*), cultures from all the lesions had a resistance ratio of greater than 256.

TABLE 1

DISTRIBUTION OF STREPTOMYCIN RESISTANCE WITHIN THE LUNGS

Case Number	Chemotherapy		Post-Streptomycin Interval Before Examination of Lung	Duration of Streptomycin Resistance Before Examination of Lung	Streptomycin Resistance of Last Sputum Culture (Resistance Ratio†)	Type of Lesion	Total Number of Lesions	Streptomycin Resistance (Resistance Ratio†)									
	Drugs*	Months						1	2	4	8	16	32	64	128	256	>256
			months	*months*													
1	PAS	7	5	Not	16	Cavities	3	—	—	—	—	1	—	1	—	1	—
	SM-(PAS)	3		known		Caseous foci	2	—	—	—	—	—	—	1	—	1	—
	(SM_1-PAS)	8															
	1 gm. Oxyt. -INH	3															
3	PAS	7	None	Not	1‡	Cavities	1	1‡	—	—	—	—	—	—	—	—	—
	SM_3-(PAS)	1½		known		Caseous foci	6	1‡	—	1	1	3	—	—	—	—	—
	SM_2-(PAS)	3															
	SM-(PAS)	2															
	(SM_3)-INH	2															
	(SM)-INH	5															
4	PAS	4	5	24	>32	Cavities	3	—	—	—	—	1	—	1	—	1	—
	INH	3				Caseous foci	2	—	—	—	—	2	—	—	—	—	—
	SM-(PAS-INH)	1															
	SM-(PAS)	2															
	(SM-PAS-INH)	1															
	5 gm. Oxyt.-(SM)	1½															
	(SM-PAS-INH)	9															
5	SM_2-PAS	7	18	21	>16	Caseous foci	7	—	—	—	—	—	—	—	—	—	7
	2 gm. Oxyt.-INH	6															
6	PAS	4	None	6	>16	Cavities	1	—	—	—	—	—	—	—	—	—	1
	INH	6				Caseous foci	5	—	—	—	—	—	—	—	—	—	5
	2 gm. Oxyt.-SM	8															

7	PAS SM-(PAS) (SM_2-PAS) (SM-PAS)-INH	10 7 3 5	6	>12	2‡	Right lung: Cavities Caseous foci Left lung: Cavities Caseous foci	 3 3 2 4	 — — — 1‡	 — — 1 1‡	 — 2 1 1	 3 1 — 1	 — — — —	 — — — —	 — — — —	 — — — —	 — — — —
10	SM-PAS SM_2-PAS 2 gm. Oxyt.-INH	6 6 5	7	10	>16	Cavities Caseous foci	2 4	— —	— —	— —	— —	— —	— —	— 1	— —	— —	2 3
11	PAS INH SM-(PAS) (SM-INH)	9 3 2 3	3	8	>16	Right lung: Cavities Caseous foci Left lung: Cavities Caseous foci	 5 11 1 2	 1 — — —	 — — — —	 — 1 — —	 — — 1 —	 1 1 — 1	 — — — 1	 — — — —	 1 1 — —	 — — — —	 2 8 — —

* SM—streptomycin; PAS—para-aminosalicylic acid; INH—isoniazid; Oxyt.—oxytetracycline. The dosage of streptomycin is indicated by the numeral after the letters SM, e.g., SM_2 = streptomycin twice weekly. SM alone indicates daily streptomycin.

The symbols in parentheses indicate that the patient's organisms were resistant to these drugs.

† By the method used in the Medical Research Council of Great Britain Trials of Isoniazid (9) using twofold dilutions of the drug in liquid medium of Davis and Dubos. The results are expressed as resistance ratios, that is, the ratio of the minimum inhibitory concentration for the test strain to the minimum inhibitory concentration for the standard H37Rv strain of *M. tuberculosis*.

‡ Resistant when tested on Löwenstein-Jensen medium (*see* text).

In 2 cases (*Cases 3* and *7*) the last available cultures from the sputum were drug susceptible when tested in the liquid medium, the resistance ratios being 1 and 2, respectively. Both of these cultures were resistant when tested on solid medium, a resistance ratio of 8 being obtained in each case. Furthermore, in each case, cultures from two lesions which were drug susceptible by the liquid medium test were shown to be resistant when tested on the solid medium. In *Case 5*, the culture from a third lesion, which was drug susceptible in liquid medium, did not grow on the solid medium test.

Comparison of the degree of resistance of cultures from cavities and caseous foci: It will be seen in table 1 that in 2 cases (*Cases 1* and *7*) there was no difference between the degree of streptomycin resistance of tubercle bacilli isolated from the cavities and caseous foci. In *Cases 6*, *10*, and *11*, no difference could be demonstrated since organisms from both types of lesions were isolated which were resistant to the maximum concentration tested. In the sixth case (*Case 4*) the maximum degree of resistance was present in cultures from a cavity. In the seventh case (*Case 3*) the maximum degree of resistance was present in cultures from a caseous focus. In the eighth case (*Case 5*) resistance tests were not available on cultures from the cavities.

Isoniazid

Drug resistance of tubercle bacilli from the sputum: The results of isoniazid-resistance tests on cultures from the sputum of the 13 patients, together with the duration of therapy with isoniazid alone or along with other drugs are shown in figure 1. When available, the results are given from the beginning of the first course of isoniazid to the time of examination of the lung specimens. The range of resistance of cultures from the lungs is also given.

Resistance of cultures from the lung lesions: In table 2 and figure 1 are shown the results of the resistance tests on cultures from the individual lung lesions. In each of the first 9 patients it will be seen that the degree of resistance of cultures from the lesions lay within three consecutive concentrations of isoniazid. This variation is inherent in the method used for detecting isoniazid resistance and, therefore, in these patients the resistance of the cultures from the lesions are considered to be uniform. In terms of the highest isoniazid concentrations permitting growth, the readings were 0.2 to 1.0 γ per ml. in one patient (*Case 6*), 1.0 to 2.5 γ per ml. in 3 patients (*Cases 1*, *2*, and *5*), 0.5 to 2.5 γ per ml. in a fifth patient (*Case 3*), 2.5 to 5.0 γ per ml. in a sixth (*Case 4*), and 10.0 to 50.0 γ per ml. in a seventh patient (*Case 7*). In the other 2 patients (*Cases 8* and *9*), the tubercle bacilli isolated from all of the lesions were resistant to isoniazid concentrations of 50.0 γ per ml., the highest concentration tested.

In the remaining 4 patients, the degrees of resistance within the lungs showed greater variation than could be accounted for by the method. In one patient (*Case 10*), the tubercle bacilli from one lesion were completely susceptible; in the other four lesions, the isoniazid resistance of the bacilli ranged from 5.0 to 50.0 γ of the drug per ml. Cultures from the lung of a second patient (*Case 11*) varied in isoniazid resistance from 0.2 to 25.0 γ per ml. The tubercle bacilli

TABLE 2

DISTRIBUTION OF ISONIAZID RESISTANCE WITHIN THE LUNGS

Case Number	Chemotherapy		Post-Isoniazid Interval Before Examination of Lung	Duration of Isoniazid Resistance Before Examination of Lung	Isoniazid Resistance of Last Sputum Cultures†	Type of Lesion	Total Number of Lesions	Isoniazid Resistance† γ per ml.								
	Drugs*	Months						0	0.2	0.5	1.0	2.5	5	10	25	50
			months	*months*	γ *per ml.*											
1	PAS	7	None	1	1.0	Cavities	3	—	—	—	3	—	—	—	—	—
	SM-(PAS)	3				Caseous foci	2	—	—	—	1	1	—	—	—	—
	(SM_2-PAS)	8														
	1 gm. Oxyt.-INH	3														
2	PAS	5	6	9	1.0	Cavities	4	—	—	—	1	3	—	—	—	—
	SM-(PAS)	$4\frac{1}{2}$				Caseous foci	1	—	—	—	1	—	—	—	—	—
	(SM-PAS)-INH	3														
	(SM)-INH	4														
3	PAS	7	$\frac{1}{4}$	3	1.0	Cavities	1	—	—	—	—	1	—	—	—	—
	SM_2-(PAS)	$1\frac{1}{2}$				Caseous foci	6	—	—	1	3	2	—	—	—	—
	SM_2-(PAS)	3														
	SM-(PAS)	2														
	(SM_2)-INH	2														
	(SM)-INH	5														
4	PAS	4	4	25	5.0	Cavities	4	—	—	—	—	2	2	—	—	—
	INH	3				Caseous foci	2	—	—	—	—	2	—	—	—	—
	SM-(PAS-INH)	1														
	SM-(PAS)	2														
	(SM-PAS-INH)	1														
	5 gm. Oxyt.-(SM)	$1\frac{1}{2}$														
	(SM-PAS-INH)	9														
5	SM_2-PAS	7	$11\frac{1}{2}$	$15\frac{1}{2}$	5.0	Caseous foci	7	—	—	—	3	4	—	—	—	—
	2 gm. Oxyt.-INH	6														

TABLE 2—*Continued*

DISTRIBUTION OF ISONIAZID RESISTANCE WITHIN THE LUNGS

Case Number	Chemotherapy		Post-Isoniazid Interval Before Examination of Lung	Duration of Isoniazid Resistance Before Examination of Lung	Isoniazid Resistance of Last Sputum Cultures†	Type of Lesion	Total Number of Lesions	Isoniazid Resistance† γ per ml.								
	Drugs*	Months						0	0.2	0.5	1.0	2.5	5	10	25	50
			months	*months*	γ *per ml.*											
6	PAS	4	21	26	1.0	Right lung:										
	INH	6				Caseous foci	4	—	1	2	1	—	—	—	—	—
	2 gm. Oxyt.-SM	8				Left lung:										
						Cavities	1	—	—	1	—	—	—	—	—	—
						Caseous foci	1	—	—	1	—	—	—	—	—	—
7	PAS	10	7	10	50.0	Right lung:										
	SM-(PAS)	7				Cavities	3	—	—	—	—	—	—	2	1	—
	(SM_2-PAS)	3				Caseous foci	3	—	—	—	—	—	—	2	1	—
	(SM-PAS)-INH	5				Left lung:										
						Cavities	2	—	—	—	—	—	—	—	—	2
						Caseous foci	4	—	—	—	—	—	—	—	—	4
8	PAS	3½	15	Not	50.0	Cavities	3	—	—	—	—	—	—	—	—	3
	INH	2½		known		Caseous foci	7	—	—	—	—	—	—	—	—	7
	SM-INH	½														
	INH	2														
	SM_2-PAS	7½														
9	PAS	4½	2	23	50.0	Right lung:										
	SM-PAS	4				Cavities	6	—	—	—	—	—	—	—	—	6
	INH	1½				Caseous foci	5	—	—	—	—	—	—	—	—	5
	(SM_2-INH)	1				Left lung:										
	(SM_2-PAS-INH)	3				Cavities	4	—	—	—	—	—	—	—	—	4
	(SM-PAS-INH)	2				Caseous foci	1	—	—	—	—	—	—	—	—	1

10	SM-PAS SM_2-PAS 2 gm. Oxyt.-INH	6 6 5	None	$1\frac{1}{2}$	50.0	Cavities Caseous foci	2 4	— 1	— —	— —	— —	— —	— 1	— 1	— 1	2 —
11	PAS INH SM-(PAS) (SM-INH)	9 3 2 3	3	Not known	10.0	Right lung: Cavities Caseous foci Left lung: Cavities Caseous foci	 5 11 1 2	 — — — —	 2 1 — —	 1 1 — —	 — 1 — 1	 — 1 — 1	 — 4 1 —	 1 2 — —	 1 1 — —	 — — — —
12	INH (INH)	4 12	6	29	50.0	Right lung: Cavities Caseous foci Left lung: Cavities Caseous foci	 4 6 3 4	 — — — —	 — — — —	 — — — —	 2 — — —	 1 1 — —	 1 — 1 2	 — 1 — —	 — 1 — —	 — 3 2 2
13	SM-PAS SM_2-PAS 5 gm. Oxyt.-INH 5 gm. Oxyt.-(INH) (SM-PAS-INH)	$3\frac{1}{2}$ 6 6 1 $4\frac{1}{2}$	7	$15\frac{1}{2}$	5.0	Cavities Caseous foci	2 7	— —	— —	— —	— 1	— 1	2 3	— 1	— 1	— —

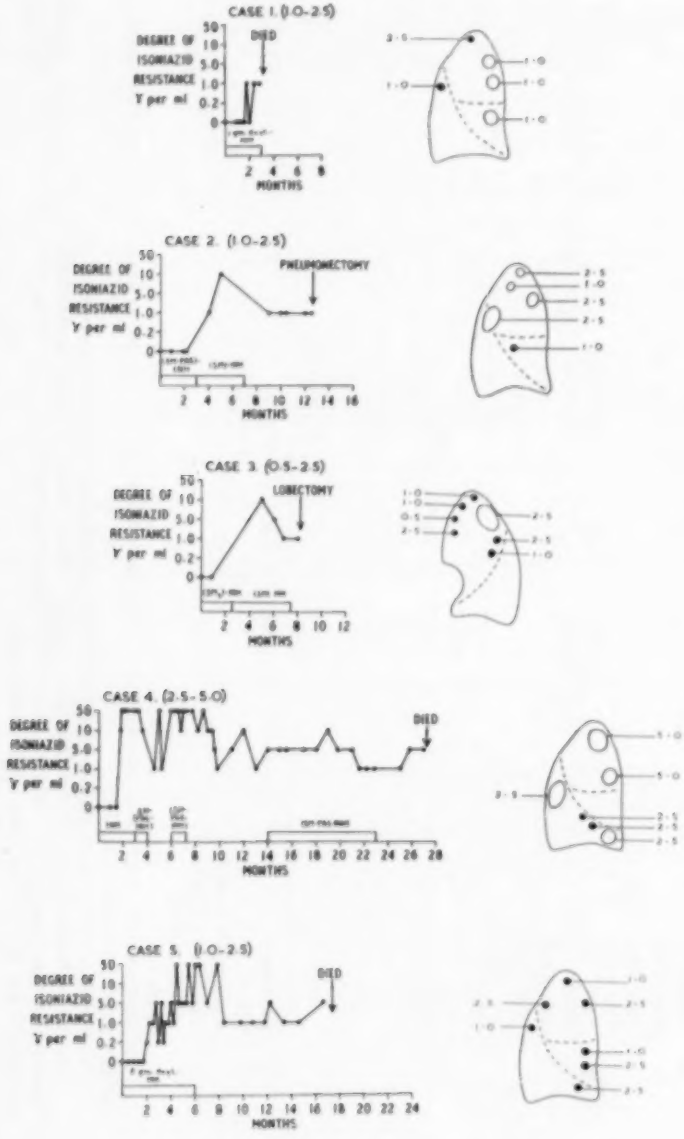
* SM—streptomycin; PAS—para-aminosalicylic acid; INH—isoniazid; Oxyt.—oxytetracycline. The dosage of streptomycin is indicated by the numeral after the letters SM.

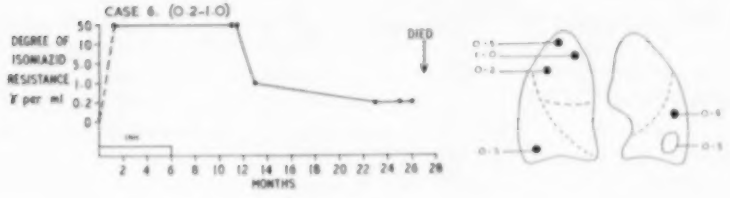
The symbols in parentheses indicate that the patient's organisms were resistant to these drugs.

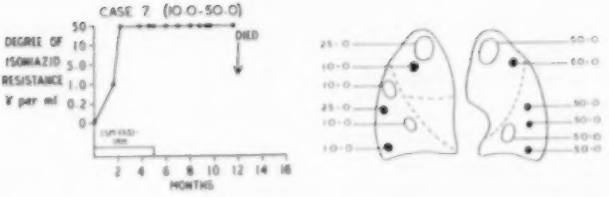
† By the method used in the Medical Research Council of Great Britain Trials of Isoniazid (9). Cultures from the sputum were tested on Löwenstein-Jensen medium containing 0.2, 1.0, 5.0, 10.0, and 50.0 γ of isoniazid per ml., and those from the lung lesions at concentrations of 0.2, 0.5, 1.0, 2.5, 5.0, 10.0, 25.0, and 50.0 γ of isoniazid per ml.

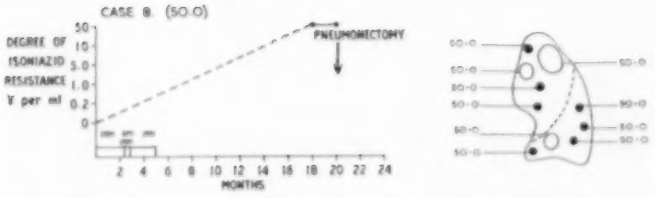
FIGURE 1

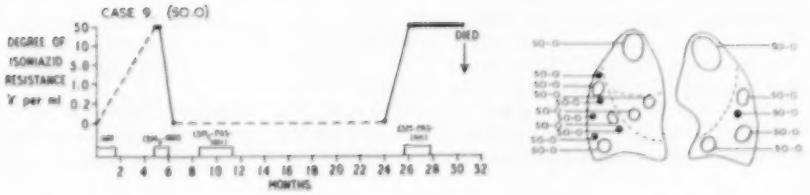
Graphs showing the result of isoniazid-resistance tests on cultures from the sputum of the 13 patients together with the duration of isoniazid therapy alone or administered along with other drugs.

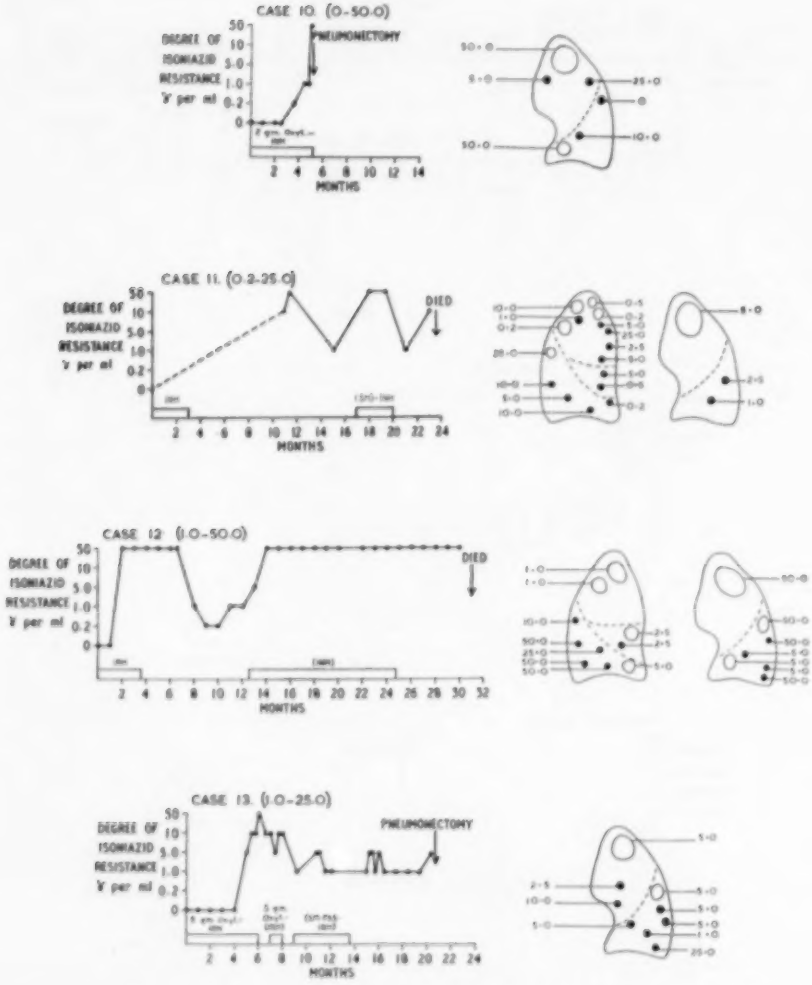



CASE 6. (0.2-1.0)
DEGREE OF ISONIAZID RESISTANCE γ per ml
50
10
5.0
1.0
0.2
0
DIED
2 4 6 8 10 12 14 16 18 20 22 24 26 28
MONTHS


CASE 7. (10.0-50.0)
DEGREE OF ISONIAZID RESISTANCE γ per ml
50
10
5.0
1.0
0.2
0
DIED
2 4 6 8 10 12 14 16
MONTHS


CASE 8. (50.0)
DEGREE OF ISONIAZID RESISTANCE γ per ml
50
10
5.0
1.0
0.2
0
PNEUMONECTOMY
2 4 6 8 10 12 14 16 18 20 22 24
MONTHS


CASE 9. (50.0)
DEGREE OF ISONIAZID RESISTANCE γ per ml
50
10
5.0
1.0
0.2
0
DIED
2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32
MONTHS



The range of resistance of cultures from the lungs is given in parentheses after the case number, and the distribution of resistance is shown in the diagrams at the end of each graph. The blocks indicate duration of therapy. The degree of isoniazid resistance is given in γ of isoniazid per ml. of Löwenstein-Jensen medium, the concentrations tested being 50.0, 10.0, 5.0, 1.0, and 0.2 γ of isoniazid per ml. The method used was that of the Medical Research Council of Great Britain Trials of Isoniazid (9). The figure 0 indicates a growth of less than 20 colonies at 0.2 γ per ml., that is, complete susceptibility.

SM—streptomycin; PAS—para-aminosalicylic acid; INH—isoniazid; Oxyt.—oxytetracycline. The dosage of streptomycin is indicated by the numeral after the letters SM, e.g., SM_2 = streptomycin twice weekly. SM alone indicates daily streptomycin.

from a third patient (*Case 12*) were resistant to isoniazid concentrations of 1.0 to 50.0 γ per ml., and in a fourth patient (*Case 13*) the degrees of resistance varied from 1.0 to 25.0 γ of isoniazid per ml.

Allowing for experimental error, the degree of resistance of the last culture of the sputum which was tested was in agreement with the maximum degree of resistance of the cultures from the lung in each of the 13 patients.

Comparison of the degree of resistance of tubercle bacilli obtained from cavities and from caseous foci: A comparison of the degree of isoniazid resistance of tubercle bacilli from cavities and from caseous foci is shown in table 2. In the 9 cases in which a uniform degree of resistance was present in the lung lesions, it was evident that no difference could occur between the degree of resistance in the two types of lesion. In 2 (*Cases 11* and *12*) of the 4 patients showing a varied degree of resistance, the maximum degree of resistance was found in both the cavities and the caseous foci. In the third patient (*Case 10*) the maximum degree of resistance was found in the cavities; and in the fourth (*Case 13*), in the caseous foci.

Comparison of the degree of streptomycin and isoniazid resistance within the same lesion: In only 2 of the patients who showed a varied degree of isoniazid resistance (*Cases 10* and *11*) were streptomycin-resistance tests also done on tubercle bacilli from the same lesions. In neither case was the maximum degree of resistance to one drug found only in cultures from lesions containing organisms with the maximum degree of resistance to the other drug.

DISCUSSION

Säenz and Canetti (4, 5) have shown that tubercle bacilli with various degrees of streptomycin resistance can be found within the same lung. Other workers (6–8) have confirmed these findings, and similar results have been found in the present series of cases.

Canetti and Säenz (5) reported that a high degree of streptomycin resistance is more often found in cavities than in caseous foci. It has not been possible to confirm this in the present study. In the Canetti-Säenz studies, the incidence of highly resistant organisms in the two types of lesion was expressed as the percentage of the total number of each lesion examined. Owing to the wide variety of chemotherapeutic regimens used in the present study, it was of little use to summate the results from all cases. Consequently, a comparison was made of the degree of streptomycin resistance of tubercle bacilli isolated from the two types of lesion in the individual lungs. In one of the 7 cases in which both cavities and caseous foci were examined, the tubercle bacilli with the maximum degree of streptomycin resistance were isolated from a cavity, but in a second case the maximum degree of resistance was found in a caseous focus. In the remaining 5 cases there was no difference in the degree of resistance found in the two types of lesion.

The finding of higher degrees of streptomycin resistance in cavities may depend on the duration of streptomycin therapy. Of the eleven cases reported by Canetti and Säenz (5) in which the duration of streptomycin therapy is

given, all but two had less than four months' treatment. In these two cases the degree of resistance of the organisms in the cavities was not higher than that of those in the caseous foci. In the present series, all of the patients had received a minimum of five months' treatment and the majority had received treatment for a considerably longer period.

It has been reported that, within the same lung, some lesions may contain tubercle bacilli susceptible to streptomycin although tubercle bacilli from other lesions may be resistant to this drug (5–7). In the lungs from six patients, all of whom had been treated with streptomycin for at least one hundred and twenty days, Fisher (11) did not find any foci containing streptomycin-susceptible organisms.

In the present series, tubercle bacilli susceptible to streptomycin by the liquid medium test were isolated from six lesions (*Cases 3*, *7*, and *11*). The cultures of bacilli from four lesions (*Cases 3* and *7*) were shown to be resistant when tested on solid medium. The remaining two cultures were not tested by this method and, therefore, it is not certain whether they were actually drug susceptible.

Organisms with various degrees of isoniazid resistance may be isolated from several lesions within the same lung (3). Such variation in the degree of isoniazid resistance was found in 4 of the 13 cases in the present series. In the remaining 9 cases, no variation in the degree of resistance was found.

There are a number of factors that may influence the uniformity of isoniazid resistance in the various lesions which merit discussion.

It has been shown that tubercle bacilli of various degrees of resistance may be present in the sputum during the development of isoniazid resistance (12). At this stage it is unlikely that the organisms within the various lung lesions would have reached a uniform degree of resistance. This is seen in *Case 10*. The marked variation in the degree of resistance present in this case may be due to one or more of the following factors:

The duration of isoniazid therapy after the emergence of resistant tubercle bacilli might influence the degree of variation of resistance within the lung. With prolonged isoniazid therapy, a uniformly high degree of resistance in the lung lesions might occur. With shorter courses of the drug, variation in the degrees of resistance might be more likely.

Different concentrations of isoniazid in lesions might result in the production of organisms of various degrees of resistance. Manthei and associates (13) have shown that different concentrations of isoniazid may be found in lesions within the same lung. In one experiment, Singh and Mitchison (14) found that, when organisms were exposed to varied concentrations of isoniazid *in vitro*, the higher the concentration of the drug, the greater the degree of resistance of the organisms which survived. These findings may explain the varied degrees of isoniazid resistance within the lung.

Schaeffer (15) has shown that isoniazid has no effect on tubercle bacilli which are not multiplying. Such dormant organisms may remain viable for a considerable period in contact with the drug and on transference to a culture medium might prove to be fully susceptible. This could explain the presence of susceptible tubercle bacilli in some lesions in spite of the existence of resistant organisms in others.

In 4 of the present cases, the tubercle bacilli in the sputum showed a constant high degree of resistance even for some months after isoniazid therapy had been stopped. In 3 of the patients (*Cases 7, 8,* and *9*), a high degree of resistance was found in all of the lung lesions. In the fourth patient (*Case 12*), marked variation in the degree of resistance was present. There is no obvious explanation for the inconsistency of these results. It might have been thought that the duration of isoniazid therapy after the development of resistance or the time since the drug was last given would influence the degree of uniformity of resistance in the tubercle bacilli in the lung. No confirmation of this supposition has been found in the particular cases studied from this standpoint.

In 5 other cases (*Cases 2, 3, 4, 5,* and *6*) sputum cultures, although at some time highly resistant, subsequently showed a constant low degree of resistance. In each of these cases cultures from all of the lung lesions showed a uniformly low degree of resistance. In another case (*Case 11*) examination of cultures of the sputum had shown that reversion to a lower degree of resistance was taking place shortly after the second course of isoniazid had been stopped. A wide variation was found in the degree of resistance of the lung cultures. One or more of the following factors might explain these results:

> If resistance to isoniazid develops by an adaptive mechanism, it might be expected that the whole bacterial population would subsequently become less resistant when no longer in contact with the drug.
>
> Mitchison has suggested that reversion of streptomycin resistance may be due to the replacement of resistant organisms by more rapidly growing susceptible ones (16). It has been shown that, within an individual lesion, organisms of low degrees of isoniazid resistance can be present although by the routine susceptibility test the culture is highly resistant (12). If organisms of a low degree of resistance grow more rapidly than highly resistant strains, the more resistant bacilli may ultimately be replaced by the less resistant ones after the patient has stopped isoniazid therapy. When completely susceptible organisms remain at the end of treatment, reversion to complete susceptibility could occur.

It is difficult to explain the results in 2 of the cases (*Cases 1* and *13*). In *Case 1*, isoniazid resistance was of recent onset and there was uniformity in the degree of resistance among the bacilli from the separate lung lesions. This is in contrast to the findings in *Case 10* in which, although drug-resistant tubercle bacilli had likewise appeared only recently, the organisms from the lung lesions showed marked variations in the degree of isoniazid resistance. In *Case 13* there was evidence of a lowering to a constant low level of the degree of isoniazid resistance of the bacilli in the sputum. Nevertheless, highly resistant organisms were isolated from some of the lung lesions. Presumably the lesions which contained these organisms were not contributing to the sputum.

In the 4 cases in which there was a variation in the degree of resistance of the organisms in the lung lesions, there was no evidence that the degree of resistance was dependent upon the nature of the lesion. Although in one case

(*Case 10*) the maximum degree of resistance was found in the cavities, in one of the lungs from another patient (*Case 13*) the maximum degree was found among tubercle bacilli from the caseous foci.

In conclusion, it is evident that many factors might have an influence on the distribution of drug-resistant bacilli within the lung, but much further work will have to be done before the exact part each plays can be elucidated.

SUMMARY

An examination was made of the distribution of streptomycin- and isoniazid-resistant tubercle bacilli in specimens of lung tissue from 13 patients.

Marked variation in the degree of resistance of tubercle bacilli from the lesions within the same lung was found in 6 of the 8 cases examined for streptomycin resistance, and in 4 of the 13 examined for isoniazid resistance.

There was no apparent difference in the degree of streptomycin or isoniazid resistance of tubercle bacilli isolated from cavities and caseous foci.

SUMARIO

Estudios de la Distribución de Bacilos Tuberculosos Farmacorresistentes Dentro del Pulmón

En los ejemplares de tejido pulmonar procedente de 13 enfermos, hízose un estudio de los bacilos tuberculosos estreptomicino- e isoniacido-resistentes. En 6 de los 8 enfermos estudiados en cuanto a resistencia a la estreptomicina y en 4 de los 13 estudiados en cuanto a resistencia a la isoniacida, descubrióse notable variación en la intensidad de la resistencia de cultivos procedentes de lesiones del mismo pulmón.

No hubo diferencia aparente en la intensidad de la resistencia a la estreptomicina y la isoniacida en los microbios aislados de cavernas y focos caseosos.

Discútense los factores que pueden afectar la uniformidad de la resistencia a la isoniacida en el pulmón.

RESUME

Recherches sur la répartition dans le poumon des bacilles antibiotiques-résistants

Il a été procédé à l'examen de la répartition des bacilles streptomycino et isoniazide-résistants dans des pièces de tissu pulmonaire provenant de 13 patients. Des différences marquées dans le degré de résistance de colonies provenant de lésions situées dans un même poumon ont été découvertes chez 6 des 8 malades étudiés pour rechercher la streptomycino-résistance et chez 4 des 13 malades étudiés pour l'isoniazide-résistance.

Il n'existait aucune différence apparente dans le degré de la streptomycino ou isoniazide-résistance des germes isolés de lésions excavées ou de foyers caséeux.

Les facteurs susceptibles d'influencer l'uniformité de l'isoniazide-résistance sont étudiés.

Acknowledgments

The writers wish to thank Professor J. W. Crofton for his help and advice in this work; Drs. I. W. B. Grant, N. W. Horne, and W. A. Murray for allowing us to investigate their cases; Mr. A. Logan for giving us access to the surgical specimens; and Dr. A. T. Wallace who carried out many of the routine tests. We are grateful to Mr. D. Hay for technical assistance and to the nursing staff of the City Hospital, Edinburgh, for their help.

REFERENCES

(1) Pyle, M. M.: Relative numbers of resistant tubercle bacilli in sputa of patients before and during treatment with streptomycin, Proc. Staff Meet., Mayo Clin., 1947, *22*, 465.

(2) Mitchison, D. A.: Development of streptomycin-resistant strains of tubercle bacilli in pulmonary tuberculosis, Thorax, 1950, *5*, 144.

(3) Stewart, S. M.: Virulence of tubercle bacilli recovered from patients treated with isoniazid, Am. Rev. Tuberc., 1954, *69*, 641.

(4) Sáenz, A., and Canetti, G.: La streptomycino-résistance du bacille de Koch en fonction de la dose de streptomycine administrée. Une étude de 150 cas de tuberculose pulmonaire, Rev. de la tuberc., 1949, *13*, 746.

(5) Canetti, G., and Sáenz, A.: La streptomycino-résistance de souches de bacilles de Koch provenant de lésions pulmonaires différentes d'un même malade, Ann. Inst. Pasteur, 1951, *80*, 238.

(6) Medlar, E. M., Bernstein, S., and Reeves, F. C.: The demonstration of streptomycin-resistant tubercle bacilli in necropsy specimens, Am. Rev. Tuberc., 1951, *63*, 449.

(7) Armstrong, A. R., and Walker, A. M.: Distribution of streptomycin-sensitive tubercle bacilli in lungs of a "resistant" patient, Canad. M. A. J., 1949, *60*, 383.

(8) Dye, W. E., and Standley, K. W.: Streptomycin-resistant tubercle bacilli: Relative resistance of cultures isolated from different sources in patients receiving the drug, Tr. Tenth Conference on the Chemotherapy of Tuberculosis, Veterans Administration, Army, and Navy, 1951, p. 103.

(9) Medical Research Council: Laboratory techniques for the determination of sensitivity of tubercle bacilli to isoniazid, streptomycin and P.A.S., Lancet, 1953, *2*, 213.

(10) Stewart, S. M.: The detection of streptomycin resistance in tubercle bacilli, J. Clin. Path., 1955, *8*, 237.

(11) Fisher, M. W.: Fitzsimons Army Hospital's report on development of resistance, Tr. Fifth Conference on the Chemotherapy of Tuberculosis, Veterans Administration, Army, and Navy, 1948, p. 74.

(12) Stewart, S. M.: Varied degrees of isoniazid resistance within strains of tubercle bacilli from sputum and pulmonary cavities, Am Rev. Tuberc., 1956, *73*, 390.

(13) Manthei, R. W., Roth, L. J., Barclay, W. R., and Ebert, R. H.: The distribution of C^{14}-labeled isoniazid in tissues and body fluids of tuberculous patients and guinea pigs, Tr. Twelfth Conference on the Chemotherapy of Tuberculosis, Veterans Administration, Army, and Navy, 1953, p. 245.

(14) Singh, B., and Mitchison, D. A.: Bactericidal activity of streptomycin and isoniazid against tubercle bacilli, Brit. M. J., 1954, No. 4854, 130.

(15) Schaeffer, W. B.: The effect of isoniazid on growing and resting tubercle bacilli, Am. Rev. Tuberc., 1954, *69*, 125.

(16) Mitchison, D. A.: The Ecology of Tubercle Bacilli Resistant to Streptomycin and Isoniazid: Adaptation in Micro-Organisms, Cambridge University Press, London, 1953, p. 253.

TUBERCULOSIS IN INFANTS AND CHILDREN[1, 2]

A Review

JOHN S. CHAPMAN

(Received for publication May 25, 1955)

INTRODUCTION

The purpose of this paper is to review the subject of tuberculosis in the younger age groups with attention to certain persistent concepts which seem to be either erroneous or ill-founded. It also seems advisable to make preliminary mention of the therapy of certain localized forms of tuberculosis for which the methods of treatment may have to be revised in the light of current experience.

ANALYSIS OF CLINICAL INFORMATION

The cases upon which this paper is based consist of 101 patients discharged from a pediatric hospital between 1949 and 1955. In table 1 it may be seen that 56 per cent of the patients were less than three years of age at the time of admission. Part of this group were residents of the local community, but approximately one-third came from other parts of the state. It is thought they represent a fairly accurate cross-section of the tuberculosis problem in this part of the United States.

Admission to the hospital was based upon the demonstration of what was regarded as significant tuberculous disease either in the chest film or, in the case of extrapulmonary tuberculosis, upon physical examination. In addition, most of these patients presented clear evidence of clinical illness in the form of fever, weight loss, and malaise. Diagnoses were confirmed by biopsy, culture methods, or by differential clinical study.

In table 2 it may be seen that 82 patients presented unmistakable pulmonary disease. The remarkable disproportion of disease on the right side may be explained partially by the data in table 3. In this table it may be seen that eleven of the thirty-four right-sided lesions were the result of bronchial obstruction, a discovery that might be anticipated from the anatomic relations of the nodes on the right side, particularly with respect to the middle and upper lobe bronchi. Nineteen patients presented bilateral infiltrations.

The nature of pulmonary infiltrations may be seen in table 4. It is striking that cavitation was demonstrable in so considerable a number of the group. The finding of tuberculous pleuritis in association with what is commonly called "primary" disease is also worthy of notice.

It is shown in table 5 that almost 20 per cent of the children discharged had,

[1] From the Department of Internal Medicine of The University of Texas Southwestern Medical School; The Ivor O'Connor Morgan Hospital, and The William Buchanan Laboratory, Dallas, Texas.

[2] Presented before the Medical Session, as part of *Section 6A*, at the annual meeting of the National Tuberculosis Association, Milwaukee, Wisconsin, May 25, 1955.

in association with other tuberculosis, enlargement of superficial lymph nodes, the diagnosis of which was confirmed by biopsy with or without culture. Of a group of 5 of these children, in whom tonsillectomy and adenoidectomy were performed, 2 were found to have tuberculosis of these tissues. Treatment of

TABLE 1

AGE OF CHILDREN WITH MANIFEST SYMPTOMATIC TUBERCULOSIS

6 months	2
6–12 months	12
12–18 months	16
18–24 months	12
2–3 years	14
3–4 years	13
4–5 years	11
More than 5 years	21
Total	101

TABLE 2

DISTRIBUTION OF PULMONARY LESIONS

Right		
Upper lobe	15	
Middle lobe	6	
Lower lobe	13	
		34
Left		
Upper lobe	6	
Lower lobe	7	
		13
Miliary	6	
Multiple sites in both lungs	19	
		25

TABLE 3

TUBERCULOUS BRONCHITIS IN EARLY LIFE

Positive findings		
Age 3 years or less	6	
More than 3 years	5	
		11
Distribution of obstructed lobes		
Right upper	5	
Right middle	6	
Left lower	1	
		12*
Total examinations	19	

* One patient had bronchitis at two separate sites.

TABLE 4

SPECIAL CHARACTERISTICS OF PULMONARY DISEASE

Necrosis and cavity		
More than 3 years of age	5	
Less than 3 years of age	7	
		12
Pleural effusion*		
Less than 3 years of age	3†	
More than 3 years of age	5	
		8

* All but one had evident mediastinal lymphadenopathy.
† One of these was an empyema in a child of 10 months.

TABLE 5

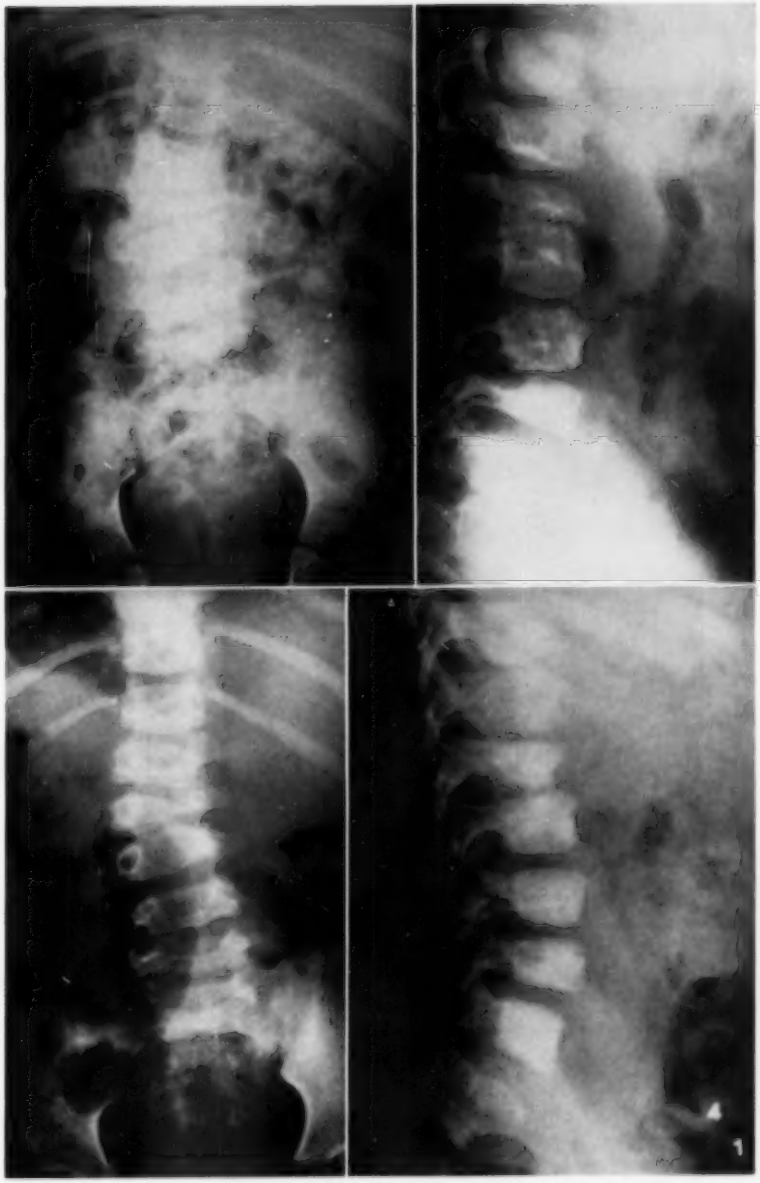
TUBERCULOUS LYMPHADENITIS IN CHILDHOOD

Cervical nodes	17	
Axillary only	2	
Generalized	1	
		20
Therapy		
Untreated	2	
Drugs only (6 short-term; 4 long-term)	10	
Drugs plus radiation	8	

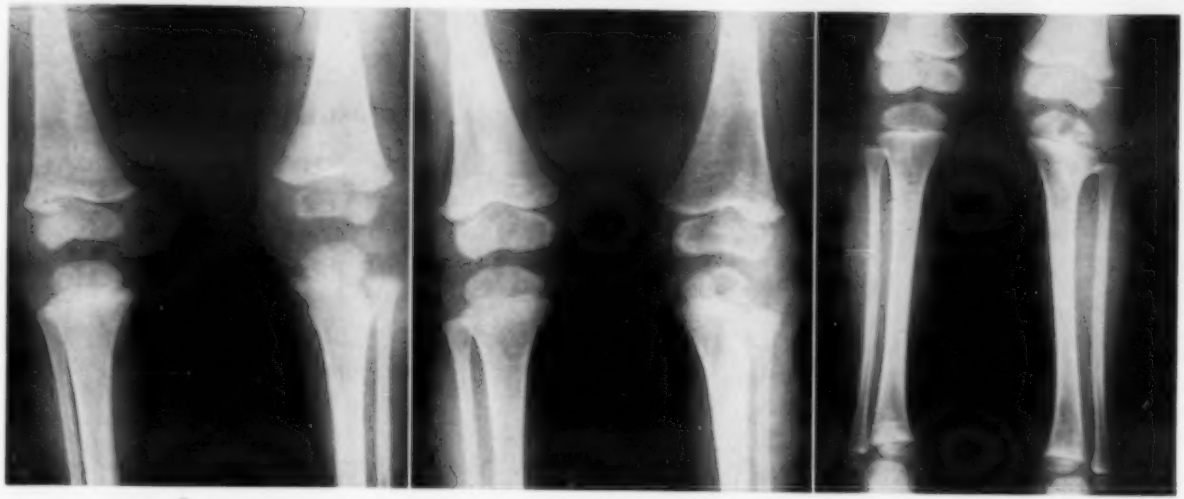
tuberculosis of the superficial lymph nodes, as will be noted, varied according to the concepts prevalent at the time. Nine of the children have been followed for a minimum of eighteen months since the beginning of treatment without evidence of recurrence. Those who were under treatment in the earlier period when drugs were used for a much shorter time have been lost to follow-up observations. The 9 satisfactory results, therefore, reflect results of long-term chemotherapy alone or in conjunction with radiation therapy.

Tuberculous osteoarthritis among these patients accounts for only a small fraction of the total hospital admissions, since children with this type of disease usually are sent to orthopedic services and are not managed medically on this pediatric service. Because of the fact that the service to which the 7 children with tuberculous osteoarthritis were admitted was a medical one, orthopedic management was comparatively limited, and 2 patients who have recently been treated with protracted courses of isoniazid-PAS are of particular interest. Figures 1–7 reveal the retrogression of the disease under this regimen. It is emphasized that these 2 children had received no significant degree of orthopedic assistance in the form of fixation, immobilization, or prevention of weight bearing. One of these patients, an infant with tuberculosis of the tibia and knee joint, now has a complete range of motion. The other child, a six-year-old Negro boy with severe Pott's disease and a very large abscess of the thigh, has comparatively little deformity and no residual abscess.

Both miliary tuberculosis and tuberculous meningitis have received consider-



FIGS. 1-4. O. S., a Negro male, six years of age, entered the hospital after seven months of dihydrostreptomycin-PAS with a very large abscess of the left thigh, which was drained surgically. Isoniazid-PAS was given from April, 1952, to September, 1953. Postero-anterior and lateral films of April 2, 1952, and August 11, 1954, are shown. There was essentially no immobilization due to the patient's destruction of body casts.



5 6 7

FIGS. 5-7. M. L. S., a white female, nineteen months old, has received isoniazid-PAS from April 20, 1954, to the present time. Aspiration of the left knee joint produced pus containing great numbers of acid-fast organisms. The right knee was swollen and tender at the time of admission. Immobilization and prevention of weight bearing occurred for not more than three months. Films of April and October, 1954, and of April, 1955, are shown.

able notice in recent literature. The writer's experience is not significantly different from that which has already been reported. Attention is called, however, to the striking difference between the results before and after the introduction of isoniazid (1). The survival of children with severe cerebral damage for an indefinite period is noteworthy, as may be seen in table 6. These children may represent a group which would have been included in the mortality figures prior to isoniazid. The only other point of interest in this particular series is that miliary tuberculosis was limited to children less than two years old and that 6 of the 21 patients with tuberculous meningitis had no other demonstrable tuberculous disease.

In table 7 may be seen once again the difference in mortality since the introduction of isoniazid. Presented also are the nature of the illness resulting in death, the days of survival following hospital admission, and the deaths in relation to age. The findings parallel those of others which indicate that the younger the

TABLE 6

FEATURES OF MENINGITIS IN INFANCY AND CHILDHOOD

With no other tuberculosis demonstrated		6
With calcific mediastinal nodes		1
With miliary tuberculosis		3
With superficial lymph node disease		2
With usual primary disease		9
Treated prior to isoniazid (4 deaths, 1 poor result)	9	
Treated with isoniazid (2 deaths, 5 poor results)	12	
Total		21

TABLE 7

ANALYSIS OF DEATHS

Total		8
Before isoniazid (of 34)	6	
After isoniazid (of 67)	2	
Miliary	2	
Miliary-meningitis	2	
Meningitis	3	
Post-meningeal hydrocephalus with aspirational pneumonia	1	

Hospital days: 3, 12, 28, 30, 45, 170, 270, 500 plus

Age of Admission	Total Discharges	Cases of Miliary TB and/or Meningitis	Deaths
1 year or less	14	6	4
1–2 years	28	11	2
More than 2 years	59	7	2

child, the higher the incidence of meningitis and miliary tuberculosis, and the worse the outlook for recovery.

TREATMENT

The chemotherapy received by the children varied with accepted practices from 1948 until approximately two years ago, at which time it was decided to establish a basic drug regimen for all. The regimen chosen consists of isoniazid, 4 mg. per kg., and PAS, 200 mg. per kg. per day, with both drugs administered for a minimum of one year.

The basis for this decision lay in the fact that cases of meningitis had been observed to develop while patients were receiving streptomycin-PAS therapy. There were also the facts that isoniazid is able to penetrate intracellularly better than streptomycin and that its limitations in the treatment of such disease as that within massively enlarged mediastinal nodes had already been defined.

In children with either miliary or meningeal infections, the practice has been to increase the dose of isoniazid to 10 mg. per kg. for the first two weeks and to give streptomycin-PAS as well. This triple-drug therapy has been continued for not less than eighteen months following the return of the cerebrospinal fluid to a normal state. Prior to the introduction of isoniazid, intrathecal streptomycin was used extensively and was followed by the sulfone, Promizole®, for a period of eighteen months to two years. None of these children received cortisone or corticotropin.

Children with tuberculosis of the superficial lymph nodes have been placed on regimens of chemotherapy according to opinion prevalent at the time. For the last four years all of them have also received radiation therapy except those whose nodes were completely removed at the time of biopsy and those who were suffering from some grave form of the disease that prohibited transportation to the therapy unit. It is the impression that nodes regressed in size more rapidly when radiation therapy was applied than when the child was under treatment with drugs alone. Radiation therapy has not been used in instances of tuberculosis of mediastinal nodes.

The treatment of osseous disease consists of isoniazid-PAS in the dosage given above. If abscesses are present, they are drained surgically. Orthopedic assistance has been rather limited.

Thoracic surgery has been necessary in 3 cases. Two resections were performed for residual cavitation and one for persistently collapsed middle lobe with disease of the bronchus in an eighteen-month-old white girl. The procedures were tolerated very well indeed. No collapse measures have been used in any of the 101 patients.

GENERAL COMMENT

The most striking difference between this group of sick children and the much larger group who are found to have no evidence of tuberculosis other than a positive reaction to tuberculin seems to lie in the fact that in the former it was almost always possible to demonstrate quite readily massive and intimate con-

tact. This suggests that, in early life at any rate, the size of the infecting dose is probably of prime importance in development of disease, while the age of the child perhaps is the most important determinant of the outcome (2). That the duration of the disease and the condition of the child at the time of hospital admission are also important is well known and is suggested again by the fact that half of the present mortality occurred within thirty days of admission. In the future the problem of the drug resistance of the tubercle bacillus may prove of equal significance.

The widespread distribution of disease in both lungs in 20 per cent of this series and the occasional occurrence of tuberculosis lying deep within the tonsils would apparently argue that there may be several sites of invasion within the same organ's system. The possibility of direct invasion through bronchial and bronchiolar walls as well as at the alveolar level has to be recognized especially since 12 patients of this series exhibited massive mediastinal adenopathy without any apparent pulmonary infiltration. But the absence of demonstrable peripheral disease might also be the result of essentially complete phagocytic removal of organisms from the site of invasion.

Tuberculosis of the superficial lymph nodes, as has been seen, is not an infrequent complication of advancing tuberculosis in the early age groups. It is well recognized that tuberculosis of the cervical lymph nodes may be the result of drainage from tonsils (3). The distribution of the nodes involved and the fact that in some cases the tonsils are not diseased suggest that other routes are important. Of these the most probable source for tuberculosis of the anterior deep cervical group seems to be the paratracheal nodes. Present knowledge of lymphatic drainage would tend to incriminate the pleura as the source of infection for the axillary groups.

The repeated occurrence of pulmonary necrosis in what seem to be the sites of invasion, the development of superficial implantation type of bronchitis, and the occurrence of effusions (4) are all points that indicate that any effort to divide tuberculosis into primary and secondary types is essentially meaningless (5) (figure 8). To one who has been familiar chiefly with tuberculosis in adults, the most striking differences consist of the location of the disease within the lung, the presence of disease of the mediastinal nodes, the marked tendency toward hematogenous dissemination, and the rather striking degree of recovery. The last named factor may be as much related to the youth of the patient as to a difference in the nature of the tissue reaction. One rather striking feature of generalized tuberculosis in early life is the absence of renal disease, a point which has been noted previously (6). In the present series, in spite of most careful search, no case of renal disease was discovered.

The bronchial lesions have already been mentioned. The pathogenesis of these lesions may be diverse. Some appear to be ordinary surface implantation disease, such as is frequently found in adults. In other instances the primary change is bronchial occlusion as a result of external compression by diseased nodes, which may even occasionally erode into the lumen (7–11) (figure 9). In some instances there is at least the possibility that with the onset of tuberculin

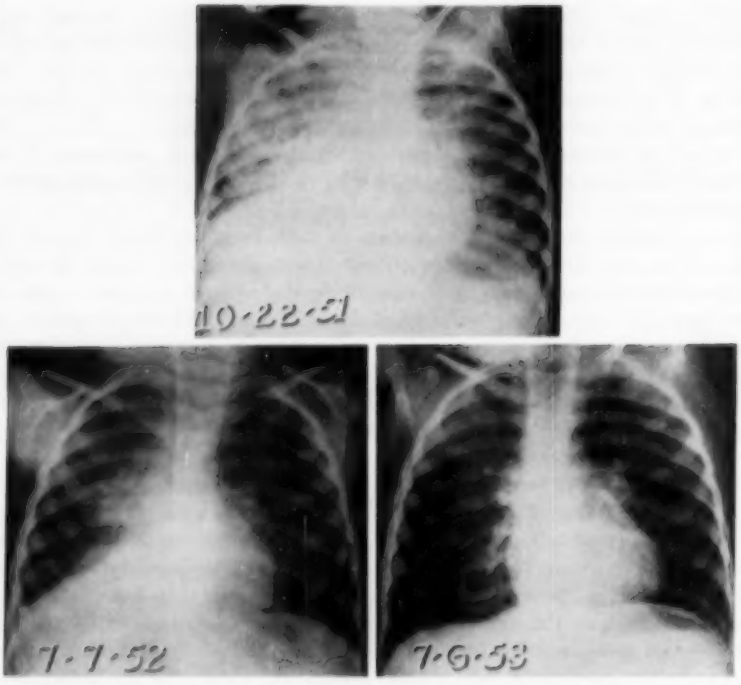


FIG. 8. L. V. was a white female, two years of age. In the films are shown both necrosis and bronchogenic dissemination to the left side. Superficial tuberculous bronchitis was also observed, which would seem to indicate the lack of a true distinction between "primary" and "secondary" tuberculosis. Complete resolution was obtained under drug therapy.

allergy the same change takes place in the bronchus that is the site of direct invasion as may occur in other sites. For the most part the impression of the bronchoscopist was that a major factor in these children was external pressure from diseased nodes. It is certainly a well-recognized fact that nodes in the mediastinum regress slowly and that the obstructed lobe or segment clears, if at all, very slowly (8, 12, 13). Indeed, pathologic studies demonstrate that many such lobes or segments are in fact quite free from any tuberculosis. This statement serves to re-emphasize the point that the condition which has been described in the past as epituberculosis is in fact no more than bronchial obstruction with a contracted and edematous or infected lobe or segment beyond (8, 12). In spite of these observations the use of the term has continued.

The rather remarkable outcome of isoniazid therapy in the 2 cases of osseous tuberculosis which have been mentioned calls for a reconsideration of the methods of management. If it is possible to preserve the function of a major joint, such as the knee, and to avoid fixation, and if this can be carried out without

jeopardizing the patient's future welfare, it would seem to be most advisable to make a more extensive study in this regard. Bosworth and others (14, 15) have already remarked upon the effectiveness of iproniazid in such a connection, and these 2 cases suggest that isoniazid-PAS over a protracted period would be equally satisfactory.

The treatment of tuberculosis of superficial lymph nodes has been dictated by the fact that radiation therapy in itself has proved to be quite effective over

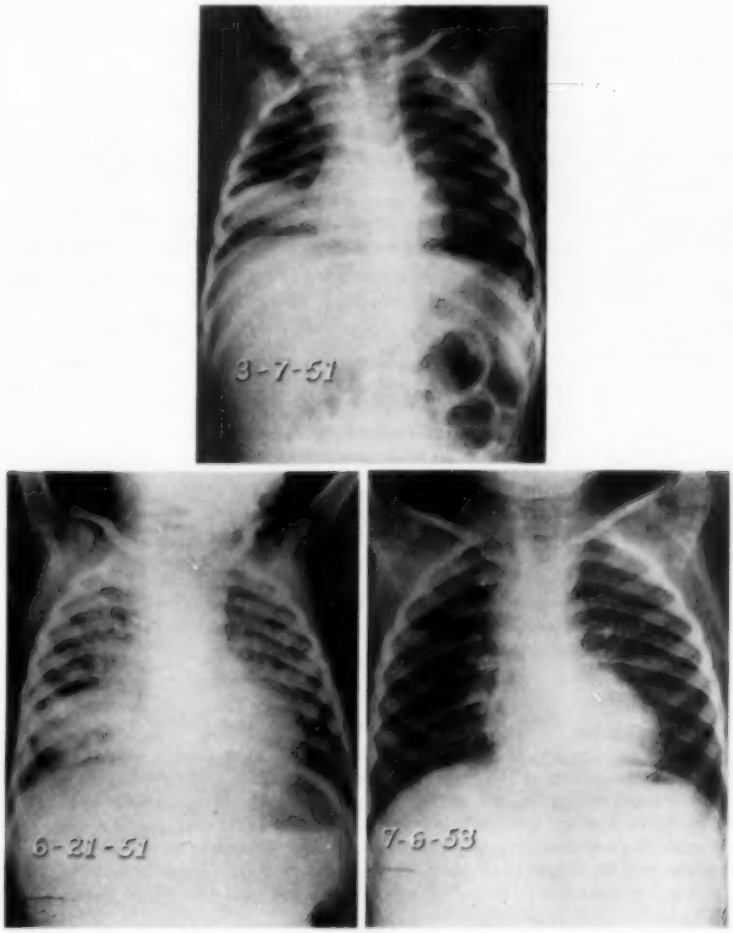


FIG. 9. R. R., a white male, aged two years, had typical occlusion of the right middle lobe associated with external compression by massive lymph nodes. Complete resolution and clearing occurred under drug therapy.

short-period follow-up in the treatment of this disease (16, 18). Nevertheless, the young child, in the face of a high probability of dissemination, requires every possible prophylaxis against hematogenous seeding (6, 10, 19, 20), and this can best be accomplished by the use of isonizaid-PAS. In appropriate circumstances, the major desideratum, whenever it is feasible and safe, is the drainage of tuberculous pus and the removal of tuberculous necrotic material (21). For this reason the treatment which has been chosen consists basically of chemotherapy of the type mentioned. To this is added excision of nodes when they are easily accessible and limited in number, the free drainage of pus when the nodes are fluctuant, and the application of radiation therapy when the nodes are multiple or when palpable masses persist after drainage (22).

SUMMARY

A group of 101 patients discharged from a tuberculosis hospital for children has been studied in relation to the character and distribution of the various types of tuberculosis encountered.

The possibility of multiple sites of invasion has been suggested. The lack of a real differentiation from tuberculosis as seen in adults has been stressed. An outline of treatment for tuberculosis of superficial lymph nodes has been suggested.

Striking results in 2 cases of tuberculous osteoarthritis are cited as offering evidence that a less drastic surgical management may be required in patients who receive adequate drug therapy.

SUMARIO

Tuberculosis en Lactantes y Niños Mayores

Un grupo de 101 enfermitos dados de alta de un hospital para niños tuberculosos ha sido estudiado en relación con la naturaleza y distribución de las varias formas de tuberculosis observadas.

Se sugiere la posibilidad de que existan múltiples sitios de invasión. Se recalca la falta de verdadera diferenciación de la tuberculosis observada en los adultos. Se sugiere un esquema de tratamiento para la tuberculosis de los ganglios linfáticos superficiales.

Cítanse los notables resultados obtenidos en 2 casos de osteoartritis tuberculosa como prueba de que, en los enfermos que reciben farmacoterapia adecuada, tal vez se requiera una asistencia quirúrgica menos fuerte.

RESUME

Tuberculose chez les nourrissons et les enfants

Après la sortie d'un hôpital de la tuberculose de l'enfance, 101 cas ont été étudiés en ce qui concerne le caractère et la répartition des diverses formes de tuberculose rencontrées.

L'auteur suggère l'existence possible de nombreux sièges d'inoculation. Le défaut d'une véritable différentiation, telle qu'elle est observée dans la tuberculose de l'adulte est mis en évidence. Une esquisse du traitement des ganglions tuberculeux superficiels est proposée.

L'auteur cite les résultats surprenants acquis dans 2 cas d'ostéoarthrite tuberculeuse prouvant qu'un traitement chirurgical moins énergique peut-être requis chez les malades soumis à une thérapeutique médicamenteuse adéquate.

REFERENCES

(1) LINCOLN, E. M.: The effect of antimicrobial therapy on the prognosis of primary tuberculosis in children, Am. Rev. Tuberc., 1954, *69*, 682.

(2) LOURIA, M. R.: Progressive primary complex, Am. Rev. Tuberc., 1940, *41*, 751.

(3) Stiefel, G. E.: Tuberculosis of tonsils and tuberculous lymphoma of neck, Schweiz. med. Wchnschr., 1953, *83*, 671 (abstracted in J.A.M.A., 1953, *153*, 1061).
(4) Ruziczka, M. W.: The course of tuberculosis in children, Wien. klin. Wchnschr., 1952, *102*, 671.
(5) Weingartner, L.: Present-day course of primary infection, Monatschr. f. kinderh., 1950, *93*, 3.
(6) Wallgren, A.: Time-table of tuberculosis, Tubercle, 1948, *29*, 245.
(7) Sailer, S.: So-called epituberculosis, Am. J. Dis. Child., 1940, *60*, 900.
(8) Miller, F. J. W.: The management of children with primary tuberculosis, Brit. M. J., 1952, *2*, 1015.
(9) Hughes, J. G., and Simpson, W. L.: Bronchiectasis following atelectasis in tuberculosis of infancy, J. Pediat., 1940, *17*, 197.
(10) Jones, E. M., and Howard, W. L.: Streptomycin in the treatment of tuberculosis in children, Dis. of Chest., 1949, *16*, 744.
(11) Gonzales, S., and Estol Baleztena, M. M.: Primary phthisis: Importance of bronchial factor, Arch. argent. de pediat., 1950, *33*, 109.
(12) Wallgren, A., and Wegelius, C.: Regression of hilar density in primary tuberculosis, Acta tubere. Scandinav., 1949, *23*, 220.
(13) Lorber, J.: Streptomycin and primary lung lesion, Lancet, 1950, *1*, 389.
(14) Bosworth, D. M., Wright, H. Q., Fielding, J. W., and Wilson, H. J., Jr.: The use of iproniazid in the treatment of bone and joint tuberculosis, J. Bone & Joint Surg., 1953, *35-A*, 577.
(15) Bosworth, D. M., and Fielding, J. W.: Chemotherapy in certain forms of juvenile tuberculosis, New York State J. Med., 1954, *54*, 2327.
(16) Aceto, J. N., Kasuga, K., and Sanderson, S. S.: X-ray therapy of peripheral tuberculous lymphadenitis, Am. Rev. Tubere., 1953, *68*, 157.
(17) Pfahler, G. E., and Kapo, P. J.: Roentgen treatment of cervical adenitis; Review of 333 consecutive cases, Am. J. Roentgenol., 1934, *32*, 293.
(18) Lampe, I., Christ, C. P., and Koch, D. A.: Concentrated roentgen therapy of cervical tuberculous lymphadenitis, Am. J. M. Sc., 1949, *217*, 632.
(19) Mouriquant, G., Boulez, N., Dechevanne, D., and Madonna, O.: Streptomycin and first infection of nurslings, Pédiatrie, 1950, *39*, 137.
(20) Bossert, O., Dude, F., and Massenberg, A.: Antibiotics and chemotherapy of tuberculosis in children, Arch. f. Kinderh., 1952, *145*, 3.
(21) MacKenzie, I. G. Chemotherapy in skeletal tuberculosis, Lancet, 1954, 1, 652 (abstracted in J.A.M.A., 1954, *155*, 933).
(22) German, J. L., Black, T. C., and Chapman, J. S.: Tuberculosis of superficial lymph nodes (to be published).

Notes

THE EFFECT OF TRIIODOTHYRONINE AND PROPYL THIOURACIL ON NATIVE RESISTANCE TO TUBERCULOSIS[1, 2]

It was shown in a previous study[3] that the phagocytes of a natively resistant rabbit respond to ingested tubercle bacilli more rapidly than those of a susceptible animal. This was indicated by their accelerated transformation into epithelioid cells and their more effective suppression of the intracellular bacteria. It was originally found in this laboratory[4] that the physiologic activity of the mononuclear phagocytes of an immunized animal is higher than that of the phagocytes of a normal rabbit, as measured by their capacity to ingest unrelated particles and their propensity to divide in response to stimuli. Suter[5] has recently found that the oxygen consumption of phagocytes obtained from an immunized guinea pig is greater than that from a normal animal. This was confirmed by Raffel.[6] Since the writers have studied the role of hormones in native resistance and since the thyroid controls the oxygen consumption of the tissues, the following experiment was performed.

Thirty litter mates of the inbred, susceptible CaC race were divided into three groups. Ten rabbits served as controls, and their basal metabolic rate was determined. Another group of 10 animals was fed 100 to 150 mg. of propyl thiouracil daily for approximately seven weeks until their pretreatment basal metabolic rate was reduced 25 per cent. A third group of 10 litter mates was given 50 γ of D-L-thyroxin intramuscularly on alternate days for two weeks and then, 40 γ of levo-triiodothyronine on alternate days by the same route for an additional two weeks. This treatment increased their initial metabolic rate by 40 per cent.

At this time the three groups of rabbits were simultaneously exposed to the quantitative inhalation of virulent human tubercle bacilli, H37Rv. The hypothyroidism of the thiouracil group was maintained throughout the course of the infection by the daily oral administration of as much as 200 mg. of this antithyroid drug and was reinforced by several subcutaneous injections of 50 mg. of this substance. The hyperthyroidism of the triiodothyronine group was continued by the intramuscular injection of 40 γ of this hormone on alternate days during the first three weeks of the infection and by daily injections thereafter.

During the progress of the infection it was evident that the tuberculin sensitivity of the hypothyroid group was markedly suppressed as compared with that of the untreated tuberculous litter mates. This was apparently due to a nonspecific anti-inflammatory effect exerted by the hypothyroid state, as the inflammatory response to india ink was also markedly reduced in these hypothyroid animals. In these circumstances, therefore,

[1] Aided by research grants from the Commonwealth Fund; the Committee on Medical Research of the American Trudeau Society, Medical Section of the National Tuberculosis Association; and the Public Health Service (Grant No. E-311(C2).

[2] The levo-triiodothyronine used in these experiments was graciously given to us by Dr. Dorsey E. Holtkamp of the Smith, Kline and French Laboratories of Philadelphia.

[3] Lurie, M. B., *et al.*: Am. Rev. Tuberc., 1955, *72*, 297.

[4] Lurie, M. B.: J. Exper. Med., 1939, *69*, 579.

[5] Suter, E.: Paper presented at the National Tuberculosis Association meeting, May 23, 1955 (in press).

[6] Raffel, S.: Bacillus and host, in *Ciba Foundation Symposium on Experimental Tuberculosis*, J. A. Churchill, Ltd., London, 1955, p. 276.

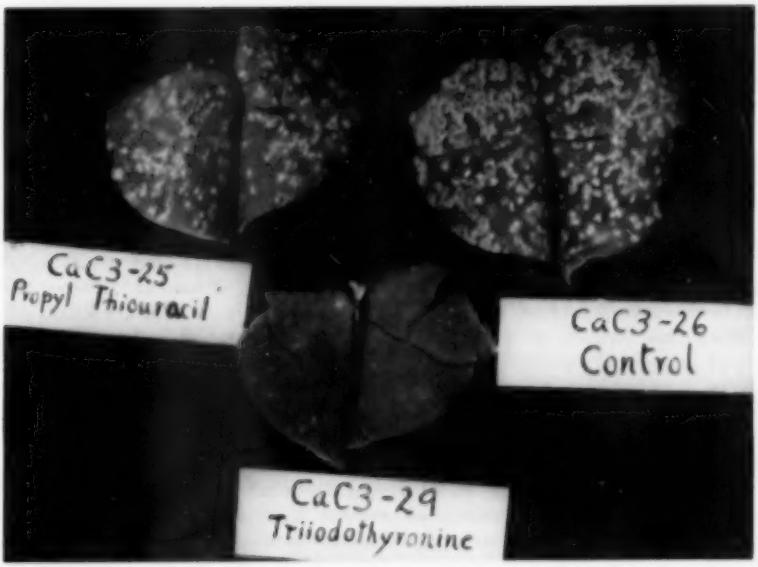


FIG. 1. The lungs of 3 litter mates born to CaC 2-67 × CaC 2-70, thirty-two to thirty-four days after the quantitative inhalation of H37Rv. Rabbits recieved an intravenous injection of trypan blue on the day before sacrifice.

CaC 3-25: propyl thiouracil; inhaled 52,600 tubercle bacilli; 473 tubercles estimated; ratio, 111; size, varies widely and averages 2.9 mm. The caseous center of most tubercles is unstained by the dye; in some the dark center indicates accumulation of trypan blue.

CaC 3-26: untreated control; inhaled 58,000 tubercle bacilli; 827 tubercles estimated; ratio, 70; size, relatively uniform and averages 3.3 mm. The caseous center of nearly every tubercle is stained with trypan blue.

CaC 3-29: triiodothyronine; inhaled 51,300 tubercle bacilli; 33 tubercles estimated; ratio, 1,555; size, 0.7 mm. There is no caseation.

thiouracil induces an anti-inflammatory effect on the tissues similar to that of cortisone. While the tuberculin reaction was much greater in the triiodothyronine rabbits than in the thiouracil animals, it was, nevertheless, considerably less than that in the controls. The probable explanation of this observation will be considered below.

Six of the hyperthyroid rabbits and 8 of the thiouracil and control rabbits, respectively, survived the experimental period, which included a heat wave. Thirty-two to thirty-five days after infection all of the animals were killed after a preliminary intravenous injection of 5 ml. per kg. of a 1 per cent saline solution of trypan blue on the day before sacrifice.

It was found that thiouracil induced a marked enlargement of the thyroid gland, whereas the administration of triiodothyronine caused a small though significant reduction in its weight. This is presumably due to the suppression of thyrotropic hormone resulting from the excess of thyroid hormone in the circulation of these rabbits. The control rabbits developed typical tuberculosis with an average ratio of approximately 100 tubercle bacilli, calculated as inhaled, yielding one tubercle. This is the ratio which was found characteristic of this race in the past. The average size of these tubercles was

3.7 mm. and each caseous center was deeply stained with trypan blue.[7] This indicated direct continuity between the circulation and the caseous center. The rabbits under the influence of propyl thiouracil showed fewer large tubercles and many small ones. The caseous center of many of these was colorless, as is true of cortisone-treated animals given an intravenous injection of the dye.[7] This confirms the above surmise that propyl thiouracil is an anti-inflammatory agent and that it interferes with the transfer of substances from the circulation into many of the caseous centers.

The most striking effects were found in the 6 surviving triiodothyronine rabbits. In 3 of these rabbits, which had inhaled about 8,000 microorganisms, no visible or palpable tubercles could be found. In each of the 3 control and the 2 thiouracil-treated litter mates, respectively, which had inhaled the same number of microorganisms simultaneously, numerous tubercles were found. The tubercles ranged in number from 11 to 209 per rabbit lung. In the remaining 3 hyperthyroid rabbits, which had inhaled 28,000 to 50,000 tubercle bacilli simultaneously with 6 untreated and 6 thiouracil rabbits, a few barely visible pinpoint- to pinhead-sized tubercles without caseation were seen. This is to be compared with the large numbers of the often extensively caseated, large tubercles in the control and hypothyroid rabbits. The tuberculosis in these three types of rabbits is illustrated in figure 1.

Histologically the tubercles in the control, untreated rabbits were compact and characteristic, with extensive, amorphous, far advanced caseous centers containing numerous long, well-stained bacilli and widespread perifocal tuberculous granulation tissue, with many mature epithelioid cells harboring occasional bacilli. In the thiouracil rabbits the tubercles were quite loose in structure and resembled, in some respects, the lesions found in cortisone-treated rabbits.[7] The intra-alveolar process predominated over the interstitial inflammation; the caseous process was retarded in its development with the persistence of numerous necrotic polymorphs and epithelioid cells which swarmed with skeins of deeply stained long bacilli. The epithelioid cells in the neighborhood of the necrotic intra-alveolar plugs were immature and contained unusually large numbers of long deeply stained tubercle bacilli. By contrast, in the triiodothyronine rabbits the tubercles were either entirely absent or extremely rare and, when found, consisted of minute collections of mature epithelioid cells in which tubercle bacilli were difficult to find, and, when seen, were very short and poorly stained. There was no caseation in them and the centrally situated epithelioid cells were flanked by round cell infiltration.

It is believed that the apparent suppression of growth of the bacilli in these triiodothyronine rabbits, and, hence, the resulting lack of antigenic stimulation, accounts for the lower tuberculin sensitivity of these rabbits as compared with that of the untreated controls, as noted above.

Many of the above preliminary observations are in harmony with those of D. A. Long and his co-workers.[8] Of particular significance is his finding that thyroxin markedly increases the production of diphtheria antitoxin in guinea pigs. It is believed that these observations justify the following hypothesis. Hyperthyroidism increases the physiologic activity of the phagocytes and thus enhances the destruction of the intracellular bacilli. Hypothyroidism may reduce the physiologic activity of these phagocytes and thus hinder the destruction of the intracellular microorganisms. It is possible that cortisone exerts its baneful influence on tuberculosis, in part, by suppressing thyroid function, as it has been demonstrated by Brown-Grant and associates[9] that the administration of cortisone

[7] Lurie, M. B.: in *Ciba Foundation Symposium on Experimental Tuberculosis*, J. A. Churchill, Ltd., London, 1955, p. 246.

[8] Long, D. A., and Shewell, J.: Brit. J. Exper. Path., 1955, *36*, 351.

[9] Brown-Grant, K., Harris, G. W., and Reichlin, S.: J. Physiol., 1954, *126*, 41.

to rabbits diminishes the release of thyroxin from the thyroid gland. Hence, the similarity in the effects of propyl thiouracil and cortisone on the tuberculous process may reflect the operation of similar thyroid-phagocyte mechanisms in the two situations.

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November 11, 1955

PSYCHOLOGICAL SIDE EFFECTS INDUCED BY CYCLOSERINE IN THE TREATMENT OF PULMONARY TUBERCULOSIS

Cycloserine is a new antimicrobial derived from one of the streptomyces.[1,2] Early in 1955 the Baltimore Veterans Administration Hospital became one of a small group of Veterans Administration hospitals to engage in a pilot study of cycloserine in the treatment of pulmonary tuberculosis as a part of the Veterans Administration-Army-Navy Cooperative Study of the Chemotherapy of Tuberculosis.

Some of the original clinical reports on the use of cycloserine in the treatment of pulmonary tuberculosis indicated that a few patients showed disturbing psychological and neurologic side effects, both depressant and excitatory in character.[1,2] Their incidence was variously observed by different investigators and had no apparent relation to dosage.[1,3] The effects ranged from depressive reactions, occasionally suicidal, to somnolence and coma; from confused mental states to convulsive seizures.

Because of these reports of untoward side effects, an investigation of such psychological reactions was initiated as part of the over-all study of cycloserine. This phase of the research was purely exploratory, being designed to identify any psychological changes attributable to cycloserine and determine their direction and intensity; and to identify the personality type or types, if any, in which such psychological reactions seem more prone to occur.

As the supply of cycloserine was limited, only 20 patients could be selected to receive it. In accordance with the provisions of the Cooperative Study protocol, it was planned that 10 would be previously untreated patients and 10 would be patients with previous antituberculous chemotherapy. The dosage of cycloserine employed was 1 gm. per day, administered orally twice daily in 0.5 gm. capsules. Occasionally this was reduced in order to control the concentration in the blood which was not permitted to rise above 30 γ per ml. The blood concentrations varied between 20 and 30 γ of cycloserine per ml. and averaged approximately 25 γ per ml. for most patients.

A control group was to consist of 10 previously untreated patients with pulmonary tuberculosis who were to receive isoniazid-para-aminosalicylic acid (PAS). Assignment to a particular treatment group was to have been by chance selection.

Because of the medical criteria set up for selection of patients, they were chosen slowly. While the first patients became available for study as early as March, not all had been chosen by August, when it was necessary to terminate the psychological section of the over-all study. Since a three-month interval was set for the test-retest procedures, no patients selected after May could be included. The limited group of 15 thus available comprised 3 previously untreated patients, 8 who were receiving cycloserine as re-treatment, and 4 "controls" who received isoniazid-PAS.

Two standard personality tests, The Minnesota Multiphasic Personality Inventory (MMPI) and the Rorschach, were administered to each patient at the beginning of the study to establish his personality base line and were administered again at its close to measure changes in the experimental (as opposed to the control) group, which might be attributed to the difference in the drug regimen, and to identify such personality types as appeared to be particularly susceptible to specific psychological reactions to cycloserine.

To evaluate more specifically mood swings and changes in behavior which reflect depression in feeling tone, two instruments were employed: the Hildreth Feeling and Attitude

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[1] Epstein, I., *et al.*: Tr. Fourteenth Conference on Chemotherapy of Tuberculosis, Veterans Administration, Army, and Navy, 1955, p. 326.

[2] Quarterly Progress Report of the Veterans Administration, Army, and Navy Study on the Chemotherapy of Tuberculosis, 1955, Volume X, Numbers 1 and 2.

[3] Mulinos, J. M.: Informal report on toxicity of JN-21, supplied by Commercial Solvents Corporation.

Scale[4], which was checked by each patient twice weekly for a period of ten weeks in order to provide a subjective description of mood level; and the Multidimensional Scale for Rating Psychiatric Patients[5], which was filled out weekly during the same period for each patient by the head nurse of the ward.

The Minnesota Multiphasic Personality Inventory: Of the 15 patients who had completed the required three months of treatment by the time the second MMPI was administered, 4 had to be dropped from the group. One patient, dying of carcinoma, was too ill to be re-tested; another died suddenly of a massive pulmonary hemorrhage before he had finished filling out the repeat questionnaire; and 2 patients refused to cooperate.[6] Thus, only 7 experimental patients and 4 control subjects were available for this phase of the study.

With regard to these patients, the following observations may be made. The four validating scales were within acceptable limits for all subjects, indicating that they were honest, careful, and objective in filling out the test. Inspection of the ten clinical scales did not disclose any notable changes in the group means before and after three months of treatment. Because the differences were so slight and because of the small number of cases available, no statistical analysis of the MMPI data was undertaken.

The Rorschach: In all, 25 patients were given the Rorschach at the beginning of treatment but, as noted previously, only 15 had been on one or the other regimen for three months or more at the cut-off date of the study and were thus available for re-test. Of these, one experimental patient, as noted above, was dropped because he was moribund. Of the 14 re-tested, 4 were control subjects, 3 were previously untreated patients, and 7 had received previous chemotherapy. Statistical comparison (by a chi-square test) of the records taken before and after three months of treatment with cycloserine revealed no significant changes in any of the nine factors which were taken into account (*M*, *FM*, *FC*, (*c*), *F per cent*, *F* + *per cent*, *A per cent*, ΣC, *R*).

A few clinical observations, however, may be pertinent. In the two cycloserine treatment groups originally tested (19 patients in all), 4 patients developed psychopathologic reactions which ranged from slight to severe. Two had mild convulsive seizures of brief duration, not repeated, which were accompanied by no rise in the blood concentrations of the drug; one had an hysterical episode, following which the drug was withdrawn for a short time and was then resumed with no recurrence of the outburst; and one complained of periods of confusion. Their Rorschach profiles therefore merit particular scrutiny.

One of the patients who suffered a convulsion discontinued treatment thereafter and has since left the hospital. He was a man of thirty-nine years, who had had no previous antituberculous chemotherapy. Preceding the actual convulsion he had had prodromal symptoms on several occasions, the chief of which was the feeling that he had been hit sharply on the chin. Except that his *R* was unusually high and his *M* five times the group median of 1.0, making for marked introversion, his Rorschach scores did not diverge remarkably from the group medians of the treatment cases for the variables studied.

The other patient, age thirty, who suffered a convulsion was continued on cycloserine

[4] Hildreth, H.: J. Clin. Psychol., 1946, *2*, 214.

[5] Lorr, M., *et al*: The Multidimensional Scale for Rating Psychiatric Patients, Veterans Administration, Washington, D. C., 1953.

[6] The fact that these 2 patients took the repeat Rorschach and checked the Feeling and Attitude Scale semiweekly without demur, manifesting no measurable alteration in test performance, suggests that their refusal to repeat the MMPI cannot be attributed to personality changes induced by cycloserine but merely to general boredom and poor rapport with the examiner.

at his own insistence. In addition to one seizure, he complained of frequent feelings of haziness which were seemingly associated with the use of the drug. His tuberculosis was of long standing and he had been considered to be terminally ill. Concomitant with the administration of cycloserine, he made a weight gain of more than 30 pounds. His Rorschach profiles showed extreme constriction, with introversive trends predominating, but his scores were not notably different from the group medians.

The patient who passed through a brief hysterical episode was a man of sixty-one who had had previous chemotherapy, as he had had tuberculosis for nine years. Formerly a severe alcoholic, he has been an ardent follower of Alcoholics Anonymous for more than ten years. What was described by his physician as an hysterical outburst was precipitated by his transfer to a new ward where he was unable to adjust. His Rorschach presented the classical picture of the hysteric, and his brief excitement was probably a function of his basic personality structure rather than a result of cycloserine.

The last patient in this group, a Negro of thirty, ill for the past four years, had complained only of periods of confusion. His Rorschach again was typical of the hysteric.

Of the 25 patients to whom Rorschachs were administered at the beginning of this study, all but 2 showed marked constriction in the experience balance, together with strong introversive trends in 10 patients, an ambi-equal balance in 7. This suggests the inhibiting and encapsulating effect of prolonged illness involving separation from home and the loss of opportunity for normal social interaction.

In the remaining 6 cases, equally constricted, extraversive trends predominate. Eliminating one, which served as a control, it was found that, of the 5 patients to whom cycloserine was administered, 3 showed the classical features of an hysterical syndrome (raw color, stereotypy, neurotic shock, et cetera). It was 2 of these 3 who manifested the psychological disturbances (other than epileptiform seizures) noted above. While these are unlikely to have been due to the drug, they point up the vulnerability of hysterics to emotional strain. This suggests that their selection for certain types of experimental procedure such as this drug study may be inadvisable because it introduces an extraneous factor which may confuse the results.

With regard to the 2 patients who suffered convulsive seizures, nothing in their Rorschachs can be considered to have predictive value.

Patients' self ratings and nurses' ratings: To evaluate the reports of the emotionally depressive effects of cycloserine, information on the day-to-day behavior of patients who received this drug and of patients selected as controls was collected. This information was of two kinds, subjective reports by the patients themselves and observations of the patients by the head nurses. To standardize the collection of this information and to reduce clinical impressions to quantitative data amenable to adequate statistical analysis, two instruments developed by other investigators for this purpose were utilized, the Hildreth Feeling and Attitude Scales[4] and the Multidimensional Scale for Rating Psychiatric Patients.[5] Because the period of observation was only ten weeks it was possible to collect data on 22 patients, 17 who received cycloserine and 5 control subjects.

Semiweekly, each patient described his current mood level by checking the statements on the Hildreth Feeling and Attitude Scales which were most appropriate. These scales have been factor analyzed by Lorr and Murney[5] and, on the basis of their analysis, a score has been derived (by summing scores on the scales which loaded on their factor *A*) for a dimension extending from euphoria, high energy, and hopefulness to dejection, low energy, and hopelessness.

Once each week the head nurse rated each patient on two scales from the MSRPP, one measuring emotional responsiveness and the other measuring anxious depression.

[5] Lorr, M., and Murney, R.: J. Clin. Psychol., 1951, 7, 381.

The data so collected were subjected to analysis of variance after a design by Edwards[8] for repeated measurements on the same subject.

The following conclusions, based on these analyses, summarize the results of this part of the study having to do with the possible depressive effects of cycloserine:

> In the doses administered in this study, cycloserine did not effect any significant changes in the emotional responsiveness of the experimental patients in comparison with the control group as measured by the MSRPP.
>
> Cycloserine did not effect significant changes in the mood level of the patients receiving it as compared to the control group.
>
> Both the experimental and control groups, however, showed significant improvement in mood level over a period of time, manifesting less depression as treatment progressed.

The last conclusion is supported by statistically significant changes (in the direction of fewer, rather than more, evidences of depressive feelings), both in the ratings by the nurses and in the self-ratings of the patients themselves. This was concurrent with general clinical improvement in both experimental and control groups and hence not differentially attributable to treatment with cycloserine.

* * *

In this study the Rorschach and Minnesota Personality Inventory were administered at the beginning of treatment to 19 patients who received cycloserine and to a control group of 6 patients who received other chemotherapy. The tests were subsequently repeated for those who had completed three months of treatment. No personality changes associated with administration of cycloserine were detected, although two convulsive seizures and an hysterical episode were observed clinically, and one patient reported feelings of confusion.

The mood level and emotional reactivity of 22 patients were also studied intensively over a period of ten weeks by repeated administrations of the Hildreth Feeling and Attitude Scales and the Multidimensional Scale for Rating Psychiatric Patients. No significant changes in the emotional responsiveness of patients in the experimental group as compared to the control group were observed. There was, however, a statistically significant improvement in mood level in both the experimental and control groups which appeared in the self-ratings of the patients and also in the nurses' ratings of them.

In summary: Even in a small experimental group, the occurrence of convulsive seizures and feelings of confusion, probably related to treatment with cycloserine, was noted. However, no specific personality variables or personality patterns, detectable on the Rorschach or MMPI, appeared to be associated with these effects, nor did these tests disclose any changes in personality over a three-month interval which appeared to be related to the drug. No confirmation of the association of depressive mood changes with cycloserine, as administered here over a three-month period, was obtained; in fact, statistically reliable changes in the opposite direction were observed.

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November 18, 1955

[8] EDWARDS, A. L.: Experimental Design in Psychological Research, Rinehart and Company, Inc., New York, 1950.

[9] Present address: National Institute of Mental Health, National Institutes of Health, Public Health Service, Bethesda 14, Maryland.

EFFECTS OF PHAGOCYTIC STIMULATION ON EXPERIMENTAL TUBERCULOSIS OF GUINEA PIGS

Sir Th. Lewis considered histamine as a mediator of the defense mechanism. As a response to any injury occurring to the organism, histamine appears first in the defense process. It is known that this biologic amine increases the capillary permeability as seen by Menkin's trypan blue test. In 1947, Jansco[1] presented convincing experimental evidence that, apart from the above action, histamine may induce locally an acquired transitory phagocytic activity in the endothelial cells of the skin capillaries, as demonstrated by the india ink test, and a systemic action resulting in the stimulation of the reticuloendothelial system to an increased phagocytic activity. This observation of Jansco has been confirmed by several investigators. Recently the present writers[2, 3] reported that phagocytic activity of specific cells could be stimulated to an increased level, not only by histamine, but also by means of certain liposoluble substances like substitutes of bicyclo-decapentane. It is the intent of the writers to report similar observations at this time.

As described by Suter,[4] a peritoneal fluid can be withdrawn from the peritoneal cavity of guinea pigs which have been injected previously with a glycogen solution administered intraperitoneally. The exudate contains mainly monocytes and only a small percentage of other cellular elements. Phagocytosis of BCG by the monocytes after one hour's incubation was studied for the influence exerted by histamine, a synthetic antihistaminic substance (diphenhydramine hydrochloride) and 1,4-dimethyl-7-isopropyl-bicyclo-decapentane (BD.I). Histamine as well as BD.I, in small amounts, stimulated the monocytes to a considerably increased phagocytic activity. If amounts higher than 100 γ of histamine per ml. were added to the phagocytic mixture, histamine decreased significantly the number of BCG cells ingested by the monocytes. On the contrary, if large amounts of BD.I are added to the phagocytic mixture, the phagocytic activity is increased as well as with the smaller amounts. The synthetic antihistaminic substance in as small quantities as 1 γ per ml. inhibited phagocytic activity of most of the monocytes and decreased phagocytosis in the still functioning cells. The antihistaminic also inhibited the stimulation of phagocytosis realized with histamine or BD.I. When 10 mg. of BD.I were injected in guinea pigs once weekly during ten consecutive weeks, monocytes deriving from these animals exhibited a stimulated ingestion of BCG similar to that observed *in vitro*. If serum from BD.I-treated animals was added to monocytes of nontreated guinea pigs, stimulated phagocytosis was observed also, certainly because BD.I was present in the blood stream of the treated animals. These experiments brought us nearer to the understanding of the mechanism of action of BD.I in experimental tuberculosis.[5, 6]

Since the possibility of functional stimulation of cells belonging to the reticuloendothelial system was demonstrated with BD.I, further search for similarly acting agents was carried out. Several substitutes of bicyclo-decapentane and substances having the same physical properties (volatile oils and their components) were tested for increasing the capillary permeability and/or inducing transitory phagocytic activity of the capillary

[1] Jansco, M.: Nature, 1947, *160*, 227.
[2] Gözsy, B., and Kátó, L.: Canad. J. Microbiol., 1955, *1*, 455.
[3] Kátó, L., and Gözsy, B.: Am. J. Physiol. (in press).
[4] Suter, E.: J. Exper. Med., 1952, *96*, 137.
[5] Gözsy, B., and Kátó, L.: Canad. J. Microbiol., 1955, *1*, 161.
[6] Kátó, L., and Gözsy, B.: Canad. J. Microbiol., 1955, *1*, 622.

endothelium. It was found that several volatile oils or their components may induce the same phenomena on the capillaries as BD.I. None of the substances showed antituberculous activity *in vitro*. It was reported previously[5, 6] that BD.I had a beneficial effect on the outcome of experimental tuberculosis in the guinea pigs.

Accordingly, thirty-three volatile oils or their components, some of them inducing endothelial phagocytosis and/or increasing capillary permeability, were tested for their effect on tuberculosis of guinea pigs. The therapeutic action of subeffective or minimal effective doses of dihydrostreptomycin was compared with that of dihydrostreptomycin and simultaneously with the volatile oils or their components. Animals were infected with a human strain of tubercle bacilli (H37Rv). Treatment was instituted twenty-one days after infection and lasted for eight weeks. The animals were injected daily with 5 and 2.5 mg. of dihydrostreptomycin, respectively, and once weekly with 10 mg. of the substances to be tested. All surviving animals were killed fourteen days after treatment was ended. At autopsy, the visible macroscopic lesions were registered in a schematic representation and results of treatment were calculated, as described by Karlson and Feldman.[7] Each organ was given a rating of 0 to 4, according to the amount of gross tuberculous disease. Of the thirty-three substances, five (linalool, citronellol, ginger oil, juniper oil, and pine needle oil) increased significantly the efficacy of small doses of dihydrostreptomycin. These five substances presumably act exclusively on the host, increasing the capillary permeability and inducing phagocytic activity of the capillary endothelium. These observations brought experimental evidence that the outcome of experimental tuberculosis was more favorably influenced by acting simultaneously on the host and the parasite than on the parasite alone.

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November 7, 1955

[7] KARLSON, A. G., AND FELDMAN, W.: Ann. New York Acad. Sc., 1949, *52*, 637.

Correspondence

A PROPOSAL FOR REDUCING THE COST OF CARE OF THE TUBERCULOUS IN COUNTRIES OF LIMITED MEANS

To the Editor of the American Review of Tuberculosis and Pulmonary Diseases:

In Latin America, Asia, and Africa, tuberculosis still ranks foremost among disabling and mortal diseases demanding hospital care. The population of these parts of the world is estimated at one and two-third billion; their tuberculosis death rates lie between 50 and 100 per 100,000; hence, counting from five to ten cases of active tuberculosis for each death, these continents may harbor from about 16.6 to 5 million sick who, by our standards, should be in the hospital. To provide such a number of beds, however, is impossible; they would cost many times the money available for total national health budgets.

Can these patients be cared for in other ways? To answer this question we must first ask: What is it that is to be done for or with them? We aim, first, to prevent them from infecting others; second, to treat their disease; and third, to restore them to useful activity. Do these aims need the costly mechanism of a hospital? Yes and no. Prevention of contagion and rehabilitation can be provided for more simply and inexpensively. But treatment, modern, active treatment? Adequate diagnosis, roentgenography, tomography, functional tests, bronchoscopy; and treatment, estimation of the effects of antibiotics, and surgery call for a properly manned and equipped hospital. For how long a time? For months and years? until the patient can safely earn his living and mingle with other men? Or for days and weeks? During the short time needed to establish an accurate diagnosis, to observe the effects of drugs and antibiotics; to carry out surgery and pre- and postoperative care? Say an average of a month? Perhaps.

Granted that the apparatus of a hospital is indispensable for only so short a time, what is to become of the patient after discharge? Herein lies the crux of the problem, for ordinarily he will still carry contagion, still need treatment, and be unfit to earn his living.

If, however, he were given quarters near the hospital, so that a medical and nursing staff could conveniently follow him and his family during the remaining course of his illness, then the hospital stay might be limited to the short period required for active diagnosis and treatment, medical or surgical; the hospital would be able to serve proportionately greater numbers of sick; and the patient, once medical treatment is under way or surgical operation has been overcome, could be watched in his own home by doctors and nurses and could be fed and attended by his family. Hospital expense per patient would be curtailed correspondingly.

One way of reducing costs might lie in a small, well-staffed and equipped, adequate but not luxurious hospital, with a rapid, active turnover of patients; a hospital acting as a center for a surrounding, more-or-less self-supporting cottage colony in which ex-hospital patients could be isolated and they and their families could be watched and treated.

As to organization of the colony: Most countries, however thickly populated, have government lands that they could grant to a tuberculosis colony without great outlay in cash. A plot of tillable land would attract the sick of an agricultural nation; enterprises in which families of patients and, indeed, ex-patients themselves could participate: truck-gardening, chicken-raising, dairying; various small industries: weaving, cabinet-making, pottery, rush- and mat-weaving, would make the colony partly, if not entirely, self-supporting.

According to this scheme, *the government*, local or national, would contribute: (*1*) land; (*2*) hospital construction and equipment; (*3*) medical and nursing services, hospital and domiciliary; (*4*) transportation; (*5*) construction of roads, water supply, and various other public utilities; (*6*) farm credit and building loans. *The colony* would contribute, with the aid of government loans, at first: (*a*) maintenance of public utilities: water, roads, light, power; (*b*) marketing of colony products. *The patient* himself or his family would contribute: construction of his dwelling, meeting certain specified minimum sanitary standards, aided by farm credit or building loans, if necessary; labor in the colony enterprises.

Advantages of the proposal may be set forth as follows:

1. The patient's family is kept intact; this is especially to be desired among a scattered farming population.

2. The contagious patient is isolated without restricting him to the confines of a hospital. The construction plan of the standard dwelling provides for isolation.

3. Medical service is facilitated. The closely grouped colony cottages function as an extension of the hospital itself.

4. Close connection and joint effort among the colonists aid education and hygiene; these advantages of a prolonged hospital stay are not forfeited.

5. Construction and maintenance costs of the hospital-colony are less than the costs of present hospitals and sanatoriums.

6. In spite of its reduced costs, the hospital colony can care for many more patients than the present hospital.

7. The family and not the government bears the major part of the patient's maintenance.

8. Prolonged indigency of the patient and his family are avoided; the colony offers them opportunities of keeping themselves financially independent.

Plans for a hospital-colony like the one proposed have been elaborated and approved by the Mexican National Security Administration (Instituto Mexicano de Seguro Social) (Eloesser, L.: Rev. mex. de tuberc., 1954, *15*, 296). The writer will be glad to furnish interested persons with further details of organization, construction, and finance.

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L. Eloesser

June 27, 1955

Books

ANTIBIOTIKA-SIBEL: INDIKATION UND ANWENDUNG DER CHEMOTHERAPEUTIKA UND ANTIBIOTIKA. By A. M. Walter and L. HEILMEYER. Pp. 813, Georg Thieme Verlag, Stuttgart, Germany, 1954, Price, DM 79.

This "primer" is a book of almost 800 pages of actual text, parts of which are in very small print, with numerous tables and graphs. It contains information concerning indication, application, dosage, efficacy, toxicity, and therapeutic results of antimicrobial drugs ranging from the sulfonamides to the tetracyclines. In addition, other treatment methods are discussed, with pertinent indications, e.g., surgical therapy of chest diseases and topical enzymatic therapy.

In a "general part" of 206 pages, the principles of antimicrobial therapy are discussed and six chapters are alloted for detailed information of sulfonamides, penicillin, streptomycin, dihydrostreptomycin, tetracycline, chlortetracycline, oxytetracycline, chloramphenicol; and of drugs with "limited" efficacy (erythromycin, carbomycin, bacitracin, tyrothricin, polymyxin, viomycin, and fumagillin). This section is concluded with a discussion of multiple drug therapy, survey tables, and references to the literature.

Examples of the usefulness of the tables in this section include the surveys of "Absorption, Diffusion and Excretion," "Side Reactions," "Chemical and Physical Properties of Antibiotics," and "Applications." In a chapter on laboratory controls, bacteriologic methods, drug-susceptibility tests, and correlations with therapeutic results are discussed and tabulated.

The second section, "Clinical Part" (578 pages) contains chapters on infections in babies and newborns, tropical diseases, infestations, the respiratory tract, tuberculosis, the digestive and urogenital tracts, the meninges, septic characteristics, surgery, gynecology, and obstetrics, otorhinolaryngology, ophthalmology, dermatology, and venereal diseases.

The chapter on the treatment of respiratory diseases (24 pages) contains a general section on bacteriology and the different antimicrobial drugs used in aerobic and anaerobic, coccal, and bacillary infections. Aerosol therapy is emphasized. In a special section, infections of the pharynx, larynx, trachea, and bronchi, as well as bronchiectasis, bronchopneumonia, and lobar pneumonias, emphysema, lung abscess, and gangrene are separately discussed. Mycoses are dealt with in the chapter on miscellaneous infections, e.g., tropical diseases, et cetera.

The discussion of tuberculosis (30 pages) contains data on the specific antituberculous properties of the drugs, including isoniazid, para-aminobenzoic acid, amithiozone, viomycin, oxytetracycline, and various combinations of the drugs. Modes of application, daily dosage, and treatment schemes are also discussed. The treatment of the following special situations is presented in detail: meningeal, miliary, primary, pulmonary, and serosal tuberculosis; and all forms of extrapulmonary tuberculosis.

Although it is understandable, it is nevertheless regrettable that the authors refrain from giving a detailed report of the treatment of pulmonary tuberculosis. They plead the necessity of far-reaching individualization and refer the reader to original papers. There is, however, a great deal on this subject in the tables and in other chapters.

This book can be of considerable practical value to practitioners and specialists provided, of course, that they have a working knowledge of German. Incidentally, the language is clear and concise. A few criticisms may be permitted. Although some non-

German, especially American, proprietary preparations are mentioned, a more generous inclusion of foreign products would increase the value of the book considerably. As cited above, very small print is occasionally used. A minor defect is the absence of an index of authors and some inconsistency in regard to alphabetic arrangement of references. In this connection, it should be stressed that the foreign literature is usually adequately quoted.

In conclusion, the reviewer believes that certain differences between German and American treatment methods must, of course, be expected. Some examples of this were accordingly found. The authors, however, are sufficiently objective in giving various points of view. On the whole, this nicely set up "primer" can well be recommended.

K. Steiner
New York, New York

Rontgenanatomische Grundlagen der Lungenuntersuchung. Third Edition. Pp. 335, 335 illustrations, Akademiai Kiado, Budapest, Hungary, 1955.

This is a large, handsomely produced radiologic-anatomical atlas of the lungs. The wealth of illustrations is designed to mesh anatomical and roentgenographic cadaver studies. Thoracic sections in all planes and at varying depths are illustrated by photographs and drawings, by roentgenograms of the cadaver sections and by tomograms. Particularly well done are colored line drawings of the anatomy of the bronchi and blood vessels which produce an excellent three dimensional effect. The vessels are studied by roentgenograms of cadaver vascular injections, but not by *in vivo* angiocardiography. A lengthy section on segmental anatomy is particularly well done, although here as elsewhere the quality of the roentgenographic illustrations lags behind that of the anatomical specimens and line drawings. Relief roentgenographic prints are used extensively in the section devoted to tomography, without obvious benefit. The literary style belongs to the less obfuscatory school of German scientific writing.

Mark Ravitch
New York, New York

The Therapy of Skin Tuberculosis. Translated and Revised by Ernest A. Strakosch. Pp. 247, American Lecture Series. Charles C Thomas, Springfield, Illinois, 1955, Price, $6.75.

The senior author, Dr. Gustav Riehl, is Professor of Dermatology at the University of Vienna and Director of the Lupus Institute of Vienna. Consequently, he has had extensive first-hand knowledge of the treatment of skin tuberculosis, particularly lupus vulgaris. The entire text is devoted to treatment methods and the greater proportion deals with lupus vulgaris. The other types of skin tuberculosis and the tuberculids are poorly covered.

The chapter on Vitamin D (calciferol) therapy is thorough and includes historical background, treatment techniques and results, contraindications, toxic manifestations, and theoretical considerations.

Isoniazid therapy, although labeled the treatment of choice, is covered in less than seven pages. In the author's experience with isoniazid for the treatment of 159 cases of lupus vulgaris, 38 cases of erythema induration, 18 cases of colliquative tuberculosis colli or scrofuloderma, and 7 cases of ulcerative mucous membrane tuberculosis, therapeutic results were good. No serious toxic manifestations were noted, although in 10 per cent of the cases treated, vertigo, constipation, headache, paresthesias or nervousness were noted. Three cases of lupus miliaris disseminatus were treated without therapeutic effect.

Chemotherapy with various drugs such as amithiozone, streptomycin, para-aminosalicylic acid (PAS), and "Kresulfin" are discussed.

The chapter on physical medicine contains a more comprehensive discussion of light therapy with the Finsen or Kromayer type of lamp than is available in American texts.

The text is unillustrated except for seven plates showing cases of lupus vulgaris before and after treatment.

One of the limitations of the book is the lack of a chapter defining the various types of tuberculosis or the methods of choice in treating the various types.

The bibliography is extensive, particularly with respect to European literature, and should prove quite valuable to anyone doing research in skin tuberculosis.

DOUGLAS TORRE
New York, New York

BOOKS RECEIVED

THORACIC SURGICAL MANAGEMENT. Second Edition. By J. R. Belcher and I. W. B. Grant. Pp. 216, Baillière, Tindall and Cox, London, England (The Williams & Wilkins Co., Baltimore, Maryland, American representatives), 1955, Price, $4.75.

LA TUBERCULOSIS PULMONAR EN LOS ENFERMOS MENTALES. By Mario Pastor Balcazar. Pp. 20, Sociedad Peruana de Tisiologia, Lima, Peru, 1955.

ATLAS OF BRONCHIAL LESIONS IN PULMONARY TUBERCULOSIS. By C. Dijkstra. Pp. 128, Charles C Thomas, Springfield, Illinois, 1955, Price, $11.00.

MEDICAL RESEARCH. A MIDCENTURY SURVEY. Volume 1. American Medical Research: In Principle and Practice. By The American Foundation. Pp. 765, Little, Brown and Company, Westbrook, Connecticut, 1955, Price, $15.00 (includes Volume 2).

MEDICAL RESEARCH. A MIDCENTURY SURVEY. Volume 2. Unsolved Clinical Problems: In Biological Perspective. By The American Foundation. Pp. 740, Little, Brown and Company, Westbrook, Connecticut, 1955, Price $15.00 (includes Volume 1).

ANUARIO DE EPIDEMIOLOGIA Y ESTADISTICA VITAL. AÑO 1951. Volume 1. By Ministerio de Sanidad y Asistencia Social. Pp. 353, Ministerio de Sanidad y Asistencia Social, Caracas, Venezuela, 1954.

ANUARIO DE EPIDEMIOLOGIA Y ESTADISTICA VITAL. AÑO 1951. Volume 2. By Ministerio de Sanidad y Asistencia Social. Pp. 899, Ministerio de Sanidad y Asistencia Social, Caracas, Venezuela, 1954.

American Trudeau Society

Medical Section of the National Tuberculosis Association

NOTICES

NEWLY ELECTED OFFICERS OF STATE AND REGIONAL TRUDEAU SECTIONS

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SECOND INTERNATIONAL CONGRESS OF MEDICAL RADIOPHOTOGRAPHY

The Second International Congress of Medical Radiophotography, which will deal with all of the medical, technical, and organizational aspects of radiophotography, will be held in Paris on April 4 to 7, 1956.

The detailed program may be obtained on request from the Secretariat, 66 Boulevard Saint Michel, Paris VI^{ème}, France.

TENTH INTER-AMERICAN CONGRESS OF THE PAN AMERICAN MEDICAL ASSOCIATION

The Tenth Inter-American Congress of the Pan American Medical Association will be held in Mexico City, April 15–21, 1957. The scientific sessions will begin on Monday, April 15, at 9 a.m.

The president of the Association is Dr. Pedro A. Gutierrez Alfaro, Minister of Sanitation and Public Welfare of Venezuela, and the executive director is Dr. Joseph J. Eller, 745 Fifth Avenue, New York 22, New York.

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1. Burrascano, J. J.: Sea View Hosp. Bull. *15*:149 (July) 1955.

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1. Wolfson, W. Q.: Mississippi Valley M. J. 77: 66, 1955.

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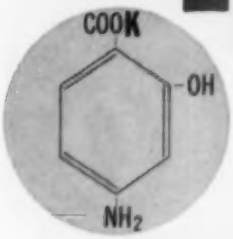

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1. *Meltham, L., Cohen, R. V., and Zarafonetis, C. J. D.: Clinical use of potassium para-aminosalicylate (KPAS). Am. Rev. of Tuberc. and Pulmon. Dis. 71:220 (Feb.) 1955.*

2. *Cohen, R. V., Meltham, L., and Zarafonetis, C. J. D.: Clinical Studies of Various Forms of PAS (with special reference to plasma concentrations). Read before the American Trudeau Society, Annual Meeting, Milwaukee, Wisconsin, May 23-27, 1955.*

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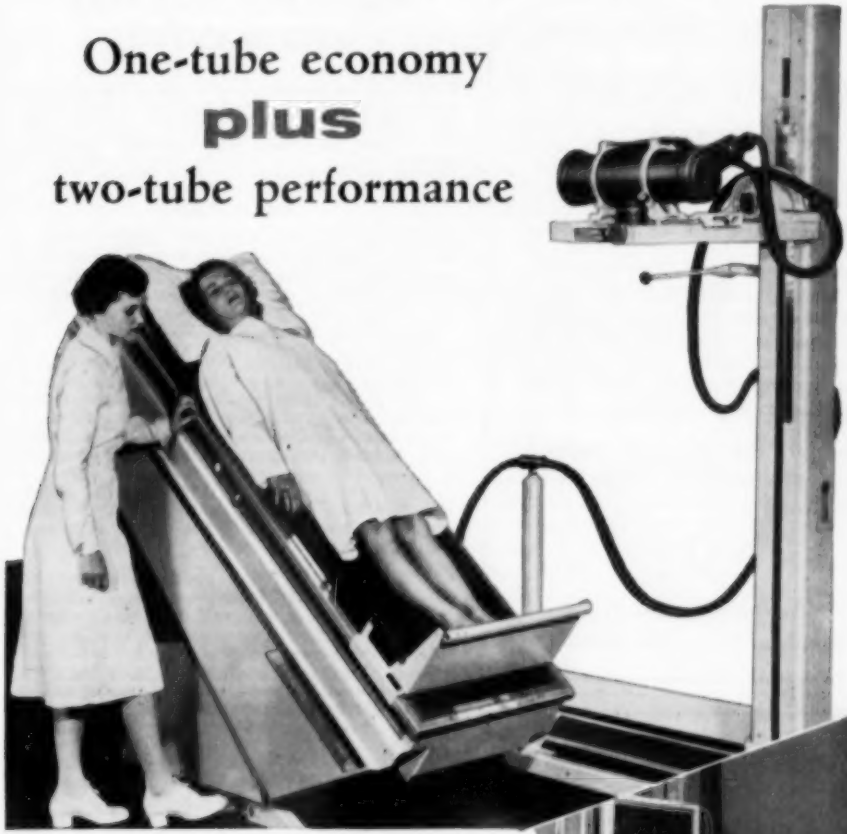

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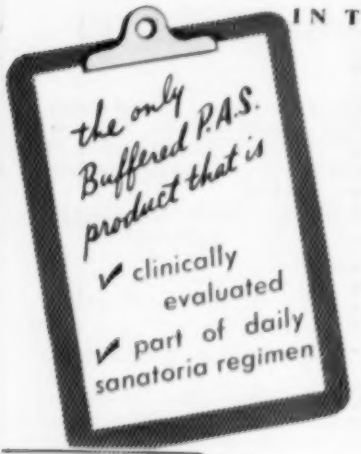

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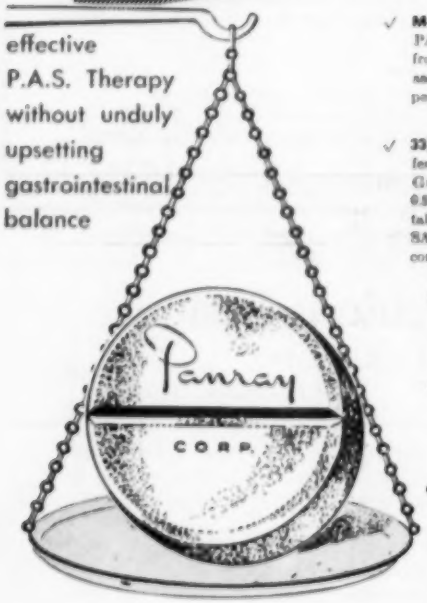

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1. Hyman, S., and Rosenbaum, S. H.: Illinois M. J. 104:297, 1953.
2. Piper, C. E., and Nichlas, F. W.: Indust. Med. 23:530, 1954.

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